ENDOCRINOLOGY

VOLUME 32 JUNE, 1943 NUMBER 6

EFFECTS OF LARGE DOSES OF ANDROGEN ON THE TESTIS IN THE GROUND SQUIRREL, CITELLUS TRIDECEMLINEATUS[1]

L. J. WELLS

From the Department of Anatomy, University of Minnesota
MINNEAPOLIS, MINNESOTA

BECAUSE of clinical as well as theoretical interest in the effects of steroidal hormones on the gonads, it seems desirable to contribute the present observations, especially since they cover a considerable range of dosages. A brief account of this investigation has been published (Wells, 1942a).

The literature on this subject has been considered recently by Moore and Morgan (1942). In several species, it has been observed that injection of androgens causes a decrease in the size of the testis. The usual interpretation has been that this was due to the inhibiting action, via the hypophysis, on the seminiferous tubules. The evidence for such action has been secured chiefly from experiments involving prepuberal animals; however, the writer knows of no instances in such animals in which injections have prevented indefinitely the production of spermatozoa. In adult animals, there are no reports that androgen reduces appreciably and consistently the number of spermatozoa within the tubules. The quantity of spermatozoa in human semen has been reported to be decreased (Kenyon, 1938; Heckel, 1939, 1940, 1941; McCullagh and McGurl, 1939) and increased (Vest and Howard, 1938; Rubinstein and Kurland, 1939).

It has been assumed that instances of doubtful, or less, inhibition of the tubules in adult animals could be explained on the basis of inadequate doses of androgen. The present experiments, designed partly to test this assumption have involved quantities of androgen ranging from massive overdoses to approximately physiological doses. As will be emphasized, the germinal epithelium was not damaged seriously, if at all, and the number of spermatozoa was not reduced.

To what extent the decrease in the size of the testis in response to androgen is due to an inhibition of the interstitial cells has not been generally considered. This is readily understandable because, with methods now available, it is impossible to determine accurately the quantity of interstitial tissue. However, the present observations suggest that a great amount of this de-

Received for publication February 16, 1943.
[1] Aided by funds from the Graduate School of the University of Minnesota.

crease is due to the inhibiting action of androgen on the interstitial cells (via the interstitial-cell-stimulating hormone of the hypophysis).

METHODS

Sixty-nine ground squirrels, *Citellus tridecemlineatus*, were used in the experiments. Many of them, hereafter referred to as 'field' animals, were collected from their natural habitat a few weeks after they emerged from hibernation. The others were immature when collected in September and were kept at laboratory temperature (about 75°F.). The latter, henceforth called 'laboratory' animals, failed to hibernate; consequently at the beginning of the experiments, they were generally fatter and therefore heavier than the field animals.

Previous to injections, one testis was removed surgically from each of the 69 animals, weighed and prepared for microscopical study. The variables introduced by unilateral castration seem to be controlled adequately since all animals were operated and since those animals dying before the eighteenth post-operative day (4 controls and 5 animals receiving hormone) were excluded from further consideration (not included with the 69 previously mentioned). Systematic examinations were made for the presence of spermatozoa, parts of the fresh organs being minced in saline and examined microscopically. If spermatozoa were not observed in the tail of the epididymis, the head of the epididymis was inspected; if not in the latter, seminiferous tubules were minced and examined.

Through the use of several criteria at the time of laparotomy, it was possible to determine rather well the status of a given animal in relation to the peak of sexual activity. These criteria included the following: weight of one testis; position of the testes (abdominal, inguinal or scrotal); motility and approximate number of spermatozoa; size of accessory reproductive organs (determined by inspection or by manual palpation); degree of scrotal pigmentation.

Among animals operated on the same day, the weights of the testes were extremely variable. This was to be expected because there occurs normally a variation of approximately 2 weeks in the time when field animals reach the peak of the cycle; the peak is attained still earlier in non-hibernating laboratory animals (Wells, 1935). The animals of the present series were arranged in pairs or small groups. The animals composing the pair exhibited similar stages of the sexual cycle, similar testicular weights and somewhat similar body weights. In order to have secured such pairs having body weights more nearly equal, an unreasonably large number of animals would have been necessary. This is because, among normal males with identical body weights, the testicular weights and the stages of the sexual cycle are related to the quantity of hypophyseal gonadotropin (Wells, 1938).

Subcutaneous injections were begun one to 3 days (usually one) after laparotomy, one animal of each pair serving as a control. The injections were made daily for 18 to 20 days. Sesame oil was the vehicle for the hormones. All controls except 9 (animals 3, 9 and 11, table 1; 6 of those not included in the tables) received blank injections of sesame oil. As a precaution against cu-

TABLE 1. EFFECTS OF TESTOSTERONE ON THE TESTIS

AN. NO.	MG. DAILY	BODY WT., GM.		WEIGHT OF TESTIS, GM.		SPERMATOZOA IN TUBULES		AUTOPSY TESTIS		SEMINAL VESICLES, GM.
		LAP.	AUT.	LAP.	AUT.	LAP.	AUT.	DIAMETER OF TUBULES, μ	INTERSTITIAL CELLS	
Adult field animals; killed May 15–22										
1	0	152	182	.6095	.5647	4	4	169	+	.8649
2	15.0	152	182	.6061	.3429	4	4	159	−	2.4502
3	0	215	210	.9201	.7753	4	4	159	+	1.0656
4	10.0	195	157	.8836	.4637	4	4	145	−	4.8243
5	0	173	183	.6931	.5854	4	4	150	+	.7407
6	7.5	170	204	.7152	.4584	4	4	143	−	1.9644
7	0	168	196	.5324	.4176	4	4	168	+	1.0148
8[1]	7.5	168	185	.5327	.4036	3	3	151	−	2.7235
9	0	200	200	.9694	.8157	4	4	159	+	1.2027
10	0.5	165	195	.9954	.3540	4	2	124	⊥	1.0719
11	0	182	200	.8360	.7365	4	4	150	+	1.5205
12	0.5	200	200	.8360	.5516	4	4	159	⊥	1.4231
Laboratory animals (all except 2 were immature); killed Feb. 14										
13	0	197	205	.7236	1.0049	4	4	181	+	.1497
14	10.0	240	235	.7888	.7488	4	4	166	−	2.2272
15	0	243	237	.5642	1.2459	0	4	159	+	.0369
16	10.0	217	190	.5508	.9433	0	4	169	−	1.1778
17	0	180	210	.4534	1.0538	0	4	194	+	.0363
18	5.0	196	200	.5044	.8756	0	4	185	−	1.1558
19	0	227	255	.2995	1.4734	0	4	190	+	.0662
20	5.0	209	185	.3064	.7401	0	4	193	−	.7524
21	0	183	185	.2662	.8416	0	2	—		.0203
22	5.0	208	185	.2042	.8109	0	4	169	−	.6644

[1] Died after receiving 20 injections.

taneous absorption of hormone by the controls, each animal was caged separately. One day after the last daily injection, the animals were weighed, decapitated and autopsied.

Reproductive organs were weighed previous to fixation. By means of the same procedure as used at laparotomy, the fresh organs were examined for the presence, the number and the motility of spermatozoa. The weights of the seminal vesicles included those of the stored secretion. The testes were fixed in Bouin's fluid, sectioned (4, 8 and 20μ) and stained with Ehrlich's hematoxylin and eosin.

The effects of androgen on the testis were judged exclusively by making two types of comparisons. First, in every animal, the autopsy testis was compared with the laparotomy testis. Second, both testes (one removed at laparotomy, the other at autopsy) of an animal receiving hormone were com-

TABLE 2. EFFECTS OF TESTOSTERONE PROPIONATE ON THE TESTIS

AN. NO.	MG. DAILY	BODY WT., GM.		WEIGHT OF TESTIS, GM.		SPERMATOZOA IN TUBULES		AUTOPSY TESTIS		SEMINAL VESICLES, GM.
		LAP.	AUT.	LAP.	AUT.	LAP.	AUT.	DIAMETER OF TUBULES, μ	INTERSTITIAL CELLS	
Adult field animals; killed May 15–22										
23	0	200	201	.7045	.6391	4	4	166	+	0.7862
24	20.0	191	185	.7025	.4150	4	4	155	−	3.5215
25	0	189	190	.7514	.5764	4	4	159	+	1.0450
26	10.0	184	186	.7436	.4507	4	4	166	−	2.5774
27	0	160	190	.7203	.5347	4	*	—		0.7926
28	5.0	185	185	.7376	.3657	4	2	146	−	5.4786
Laboratory animals; killed Feb. 14										
29	0	200	210	.8779	.9316	4	4	207	+	0.1882
30	10.0	254	250	.8886	1.2345	3	4	190	−	2.0075
19	0	227	255	.2995	1.4734	0	4	190	+	0.0662
31	10.0	235	270	.3087	.9794	0	4	163	−	1.6912
32	5.0	210	240	.3186	1.1113	0	4	173	⊥	1.7624
21	0	183	185	.2662	.8416	0	2	—		0.0203
33	5.0	243	200	.2328	1.0066	0	4	191	⊥	1.4585
Laboratory animals; killed Feb. 1										
34	0	236	218	.7519	.9508	*	4	162	+	0.6612
35	0.5	188	174	.7971	.7975	*	4	169	⊥	1.1588
36	0.2	207	192	.7354	.9179	*	4	161	⊥	1.2000
37	0.1	267	268	.6910	.8347	*	4	175	⊥	0.7861
38	0	230	226	.5768	1.0319	*	4	165	+	0.5628
39	0.2	203	160	.6351	1.0496	*	4	163	⊥	1.0658
40	0	284	258	.4309	.5392	*	3	155	+	0.0119
41	0.2	174	181	.4138	.6832	*	4	166	⊥	0.7861
42	0.1	211	181	.4471	.6854	*	4	149	⊥	0.8046
43	0.05	245	227	.4161	.4028	1	2	131	⊥	0.4106
44	0	271	255	.4838	.9304	2	4	172	+	0.0317
45	0.05	213	195	.4836	.3674	*	2	137	⊥	0.5287
46	0.05	252	240	.9415	.8656	*	4	186	⊥	0.5262
47	0.05	203	198	.2604	.3485	0	2	—		0.4325

* Spermatozoa present in epididymis.

pared with the corresponding testes of its paired control; from the foregoing account, it should be obvious why it would be undesirable, for example, to consider average weights of the testes (cf. tables 1 and 2).

Measurements were taken of 25 consecutive seminiferous tubules which lay adjacent to the tunica albuginea. From such measurements, made with an ocular micrometer, the average diameter was computed.

In sections of each testis, the number of spermatozoa was estimated. The estimates are indicated in tables 1 and 2 through the use of arbitrary ratings

ranging from 0 (none) to 4 (many in most of the tubules, *i.e.*, the maximal number for normal males of this species).

The interstitial cells of the testis were studied by means of an oil-immersion objective. Since the writer knows of no satisfactory method for determining the number of these cells, the estimated number was recorced as many, several, or few. Although numerous cellular and nuclear measurements were made, it became increasingly apparent that the data thus obtained were not reliable. This is due to the great variability in the contour of the cells and their nuclei.

OBSERVATIONS

At the time of laparotomy, the field animals were approaching the peak of genital development. The testes were scrotal; the epididymides contained quantities of spermatozoa which, upon being examined in saline, exhibited motility; the size of the accessory reproductive organs was approximately ⅗ to ⅘ of the seasonal maximum; scrotal pigment was maximal. The laboratory animals exhibited lesser sexual development: in many of them (table 1, animals 13, 14, table 2, animals 29, 30 and 34–47), the testes were scrotal, the epididymides contained few spermatozoa and the scrota were not pigmented; in the others, not yet mature, the testes had not descended into the scrota and the bulbar glands could not be palpated.

During the interval between laparotomy and autopsy, field controls exhibited maximal, normal sexual development. Their testes decreased in size (accessory reproductive organs increased) but continued to produce great quantities of spermatozoa and to exhibit numerous, large interstitial cells. These polygonal cells were arranged in clusters. Their abundant cytoplasm contained many small vacuoles and eosinophilic granules. The large nuclei were globular or oval. These controls were killed just a few weeks before the reproductive systems would be expected to show unmistakable signs of seasonal involution.

Laboratory controls, previous to autopsy, did not complete their sexual development. Although spermatozoa increased in number, or were produced, the accessory reproductive organs did not reach their maximal size.

During the period of injection, the animals receiving the larger amounts of androgen[2] lost considerable and variable quantities of semen. Although ejaculations were not noted, semen was observed frequently to ooze from the penis while injections were being made. The loss between injections was indicated by the frequent presence of coagulated semen on the perineum. Variations in the amount of semen lost may account for certain unexpected weights of the seminal vesicles (see table 1, animals 2 and 4; table 2, animals 26 and 28).

By comparing the weights of the seminal vesicles, the physiological effectiveness of various doses may be judged. Approximate physiological doses were 0.5 mg. of testosterone and 0.1 mg. of testosterone propionate, amounts greater than these being physiological overdoses.

While injections were in progress, the testis of most animals remained in

[2] The hormones were furnished by the Schering Corp., Bloomfield, N. J., through the courtesy of Dr. Erwin Schwenk.

the scrotum. In the others which had an abdominal testis, descent occurred during the period of injection.

Effects of testosterone on the testis. Changes in testicular weight occurred in controls as well as in animals receiving hormone (cf. 'laparotomy' and 'autopsy' testes, table 1); hence relative changes have been utilized in analyzing the data, the controls being counted as unity. In field animals receiving testosterone, the weight of the testis decreased more rapidly than in controls, relative changes ranging from -5.9 to -1.1. In laboratory animals injected with hormone, testicular weight increased less rapidly than in controls, the relative changes varying from $+0.4$ to 1.0; in one animal (number 14, table 1), the testicular weight decreased. The relative changes were proportional neither to the amounts of hormone nor to the shifts in body weight. It seems certain that injections of testosterone exerted an unfavorable and a significant influence upon testicular weight.

The seminiferous tubules were about the same size in most of the animals. In 70 per cent of the animals receiving testosterone, the tubules were somewhat smaller than those of controls; however it is questionable whether the differences are significant. Generally the germinal epithelium did not show detectable signs of damage, all stages of spermatogenesis being present. Within the tubular lumina of treated animals, spermatozoa were approximately as numerous as in those of controls. The tubules were exceptional in one animal (number 10) which was given daily injections of 0.5 mg. and which previous to autopsy appeared to be healthy. Relatively, these tubules were small and the spermatozoa scarce, the tubules resembling those of an exceptional control which is not listed in the tables. It seems unlikely that testosterone was the causative agent since in another animal receiving identical injections (table 1, animal 12) the tubules definitely were not reduced in size.

Interstitial cells were damaged severely in all animals receiving 5 to 15 mg. of testosterone (in table 1, such damage is indicated by the rating '$-$'). Relatively they were small and scarce, and they tended to appear singly rather than in clusters. The decreased size of the cells apparently involved both cytoplasm and nucleus. The cytoplasm was very scarce. Within it, vacuoles were missing, while eosinophilic granules were rare or absent. The nuclei were extremely hyperchromatic.

In animals receiving 0.5 mg., the interstitial cells were injured less severely (rating $\perp$). The cells were smaller and less abundant than those of controls, clusters of cells being rarely encountered. Nuclear hyperchromia was moderate. The cytoplasm, relatively scarce, contained eosinophilic granules but lacked vacuoles.

Effects of testosterone propionate on the testis. In field animals injected with this hormone, the weight of the testis decreased more rapidly than in controls (table 2). The relative change in weight ranged from -4.4 to -1.7. However, among laboratory animals, the changes in testicular weight exhibited no consistent relation to the changes which occurred in controls.

Generally the seminiferous tubules were normal. The germinal epithelium exhibited all stages of spermatogenesis. There was only one animal in which the number of spermatozoa was smaller than that in the control

(animal 28). Identical quantities of testosterone propionate did not cause a reduced number of spermatozoa in two other animals (32, 33). It is possible to compare average tubular diameters of 13 experimental animals with those of suitable controls. In 69 per cent of the cases, the diameters were smaller than those of controls. It is questionable whether the differences are significant.

The interstitial cells resembled those of animals receiving testosterone but it seems that they were affected less by testosterone propionate than by testosterone. Severe cellular damage was noted in 5 of the 7 animals which were injected with 5 mg. or more (rating −). In animals receiving less than 0.5 mg., the cells were perceptibly, although mildly, damaged (rating ⊥).

DISCUSSION

Judging from the testicular weights, injections of testosterone inhibited the testes in the majority of instances. Injections of testosterone propionate produced a similar effect in a much smaller percentage of cases. The question arises as to what components of the testis were responsible for the smaller weights. Undoubtedly the interstitial cells were involved to a great extent, they being injured in all cases; in mice and rats the inhibition of these cells has been noted previously (Selye and Friedman, 1941; Shay, Gershon-Cohen, Paschkis and Fels, 1941). But it is unlikely that the more extensive reductions in weight were due solely to the interstitial cells. Indeed in one animal (no. 30, table 2) severe injury of the cells was associated with no decrease of the weight of the testis as a whole. Also in another animal (no. 10, table 1) the testis and its seminiferous tubules were especially small, while the interstitial cells were only mildly damaged. Consequently it seems probable that the reduced weights were related to the size of the tubules. In more than half of the animals receiving hormones, tubular measurements were somewhat smaller than those of controls.

When the tubules were smaller, it is possible that this was due to the exceedingly rapid release of spermatozoa rather than to an inhibiting action of androgen. This view is supported by the fact that the testes of normal ground squirrels become smaller during the time when spermatozoa are becoming more numerous (Wells, 1935).

That the seminiferous tubules were not damaged seriously, if at all, is indicated by the following observations. The germinal epithelium presented a normal appearance; the spermatozoa were approximately as numerous as in the controls. If the tubules had been inhibited, it would be rather difficult to interpret such an effect because in this species injections of androgen may induce the formation of spermatozoa (Wells, 1942b, 1943).

In view of the wide range of dosages and especially of the larger ones, the failure of androgen to inhibit spermatogenesis is particularly noteworthy. The largest dose, 20 mg. per day, was approximately equal to that currently used therapeutically in man, yet the animals were very small in comparison with man. Even 5.0 mg. per day of testosterone propionate caused the seminal vesicles to be 3.6 times heavier than those which occur normally at the peak of the sexual cycle (cf. animal 11, table 1 and animal 28, table 2). In several

adult animals receiving overdoses of testosterone or testosterone propionate, semen actually oozed continuously from the penis for many days preceding autopsy. Yet, in spite of such excessive androgenic stimulation, the germinal epithelium was not injured perceptibly and the spermatozoa were very numerous. In immature ground squirrels, overdoses did not prevent the formation of large quantities of spermatozoa; neither did the smaller doses. In rats, it has been reported that only small doses decrease the weight of the testis (Selye and Friedman, 1941; Shay, Gershon-Cohen, Paschkis and Fels, 1941) and that large doses maintain the testis at a normal size (Greene and Burrill, 1941).

The present observations suggest that in the ground squirrel injections of androgen depressed the interstitial-cell-stimulating hormone of the hypophysis. If the hypophyseal gametogenic hormone was inhibited by androgen, the inhibition was not severe enough to cause detectable damage to the germinal epithelium.

SUMMARY AND CONCLUSIONS

Sixty-nine ground squirrels (*Citellus tridecemlineatus*) most of which were adults were used in the experiments. Injections of testosterone or testosterone propionate were begun shortly before the peak of the annual sexual cycle or, in some cases, while the animals were immature. The daily dose ranged from 0.05 to 20.0 mg. and injections were made for 18 to 20 days. Controls received blank injections.

Judging from testicular weights, the testes were inhibited in a majority of the cases. The interstitial cells of the testis were severely injured. The germinal epithelium did not show signs of damage. The number of spermatozoa was not reduced. In immature animals, the formation of spermatozoa was not prevented.

It appears that the interstitial-cell-stimulating hormone of the hypophysis was inhibited. If the hypophyseal gametogenic hormone was depressed, the depression was so slight that detectable changes in the germinal epithelium did not occur. It is unlikely that the failure of androgen to inhibit spermatogenesis was due to inadequate doses.

REFERENCES

GREENE, R. R., AND M. W. BURRILL: *Endocrinology* 29: 64. 1941.
HECKEL, N. J.: *Proc. Soc. Exper. Biol. & Med.* 40: 658. 1939.
HECKEL, N. J.: *J. Urol.* 43: 286. 1940.
HECKEL, N. J.: Tr. Am. A. *Genito-Urin. Surgeons* 34: 237. 1941.
KENYON, A. T.: *Endocrinology* 23: 121. 1938.
MCCULLAGH, E. P., AND F. J. MCGURL: *J. Urol.* 42: 1265. 1939.
MOORE, C. R., AND C. F. MORGAN: *Endocrinology* 30: 990. 1942.
RUBINSTEIN, H. S., AND A. A. KURLAND: *South. Med. J.* 32: 499. 1939.
SELYE, H., AND S. FRIEDMAN: *Endocrinology* 28: 129. 1941.
SHAY, H., J. GERSHON-COHEN, K. E. PASCHKIS AND S. S. FELS: *Endocrinology* 28: 485. 1941.
VEST, S. A., AND J. E. HOWARD: *J. Urol.* 40: 154. 1938.
WELLS, L. J.: *Anat. Rec.* 62: 409. 1935.
WELLS, L. J.: *Endocrinology* 22: 588. 1938.
WELLS, L. J.: *Anat. Rec.* 82: 479. 1942a.
WELLS, L. J.: *Anat. Rec.* 82: 565. 1942b.
WELLS, L. J.: *Surgery* (in press). 1943.

ABILITY OF ADRENAL CORTICAL HORMONES, PROLACTIN AND THYROXIN TO SUSTAIN WEIGHT OF BODY AND VISCERA OF HYPOPHYSECTOMIZED PIGEONS

RICHARD A. MILLER AND OSCAR RIDDLE

From the Department of Genetics, Carnegie Institution of Washington
COLD SPRING HARBOR, NEW YORK

AN EARLIER study by Schooley, Riddle and Bates (1941) showed that during the 10 days following the removal of the anterior pituitary gland of pigeons there is a marked loss in body weight (20 per cent) and a notably greater loss in the weight of the intestine (43 per cent) and pancreas (54 per cent). These weight losses, except that in the pancreatic tissue, were prevented and even over-compensated by moderate daily dosage with highly purified prolactin. Maintenance of the pancreatic weight seemed to require thyrotropin in addition to prolactin. Most of the preparations of prolactin used in that study had no detectable effect in maintaining the weight of the thyroids, gonads or adrenals; three of the preparations used, apparently as effective as others, had been heated to 60°C. for 5 hours to inactivate any 'growth' hormone. Mixtures of pituitary hormones other than prolactin prevented some of the loss of weight of the body and viscera, and in addition maintained or increased the weight of gonads, thyroids and adrenals.

The main purpose of the present study was to determine whether hormones of the adrenal cortex, either alone or in combination with a suboptimal dose of prolactin, contribute to the maintenance of weight of the body and viscera in hypophysectomized pigeons. A preliminary report of these results has been published (Miller and Riddle, 1942, b). Thyroxin, testosterone and insulin have been partially investigated for a similar action. In the study of Schooley, Riddle and Bates (1941) tests on 75 birds treated with cortical preparations or desiccated thyroid in combinations with various vitamins gave inconclusive results which were not described in detail.

Both the earlier and present studies are based upon the view that adequate knowledge of the mechanism by which the pituitary affects bodily growth must include a determination in young animals of all generally favorable effects of pituitary activity, such as increased food intake and increased weight of the body and of essential viscera. These favorable effects may result from actions of *a*) individual pituitary hormones and of their combinations, *b*) the hormones produced in the several glands which are stimulated by the pituitary, and *c*) these latter when combined with the various pituitary hor-

Received for publication March 1, 1943.

mones. Indeed, because of probable species differences, all of these tests should be made on several species of higher animals.

MATERIAL AND METHODS

White Carneau pigeons, aged 1.5 months (±2 days) from hatching, were hypophysectomized (anterior lobe only) and injected, in groups of 5, with one or another of the hormones tested for 10 successive days beginning on the day of operation. Most of the experimental treatments were repeated on one or more groups of birds obtained in subsequent shipments from the same commercial source. A group of uninjected hypophysectomized pigeons was kept as controls for all other birds of the same shipment. Data from 3 groups of unoperated normal animals were obtained at intervals during the course of the study; a larger number of normal birds of the same age and race which had been killed just before the initiation of the present study provided additional data. The pigeons were injected and weighed at the same time each morning. All measurements of body weight exclude food present in the crop (ingluvies). Adrenal cortex extract was injected twice daily; all other substances were given once daily.

The total amount of food and water consumed in the 10-day period was measured and the average daily consumption calculated. No value for food intake is in error by more than 2.5 gm. per bird per day. The main source of error (evaporation from container) in measurements of water consumption was present in all measurements, but losses from this source were equal only for those groups which were tested simultaneously. The maximum error for any value reported is less than 6 cc. Both actual water intake and evaporation from containers are increased in hot weather. The experimental cages were large enough to permit free movement and some flight by the birds. Completeness of hypophysectomy was verified by sectioning the fossae in doubtful cases; only data from birds which were successfully operated and which survived the 10-day period of injection are reported.

The desoxycorticosterone acetate and testosterone propionate used were crystalline products.[1] Each cc. of the adrenal cortex extract[1] was stated to be the extract from 75 gm. of fresh adrenals. Assayed by the method of Bates, Riddle and Miller (1940) the preparation of pituitary gonadotropin and thyrotropin, number 549, contained the following units per mg.: prolactin, <0.02; gonadotropin, 0.53; thyrotropin, >0.5; corticotropin, 0.16. The assay findings on the prolactin preparation, number 680, are: prolactin, 11.0 U (15.0 I.U.); gonadotropin, 0.01; thyrotropin, <0.03; corticotropin, 0.03. Three of the tabulated tests (thyroxin; FSH and thyrotropin 549; prolactin 680) were made on small groups of birds; additional and confirmatory data for these groups may be found in the study of Schooley, Riddle and Bates (1941). That study also provided data concerning the extent to which force-feeding alone maintains body and various organ weights in these hypophysec-

[1] The desoxycorticosterone acetate and testosterone propionate used in this study were contributed by Dr. Erwin Schwenk, Schering Corp., Bloomfield, N. J. Adrenal cortex extract was contributed by Dr. David Klein, The Wilson Laboratories, Chicago, Ill.

tomized pigeons. The present study has not combined force-feeding with any type of treatment.

Two types of variability, neither of which is negligible, are present in the data of table 1 and figure 3: *a*) The initial body weights of some groups of birds differ markedly (mainly a seasonal difference) from those of other groups; the organ weights obtained 10 days later are of course influenced in some degree by these differences in body weight at the time of hypophysectomy. *b*) Although space permits giving an index of variability for only four of the more significant columns of data it is notable that most tests were made at various levels of dosage and the separate recording of these tests provides further information on the reliability of the results.

RESULTS

Body weight. At the age chosen for this study the Carneau pigeons had already achieved about four-fifths of their adult weight and were growing at a diminished rate. This slow gain was at once changed to loss by removal of the pituitary; during 10 days after operation 43 untreated controls lost 21 per cent of their initial weight. Factors other than actual removal of the pituitary contributed to the decrease. Schooley, Riddle and Bates (1941) found that pigeons subjected to a 'sham' operation do not grow as rapidly as entirely normal animals. Also, a slight temporary loss of weight in unoperated control birds immediately after starting the 10-day tests (fig. 2) apparently resulted from moving the birds out of stock pens into experimental cages where their intake of food and water was measured.

Changes in the body weight of the hypophysectomized pigeons are shown in figures 1 and 2 and in table 1. Animals injected with adrenal cortex extract lost less weight (14 per cent of initial weight) than the operated controls (21 per cent); this loss was progressive throughout the 10-day period (fig. 1). Doses of 1 to 3 mg. of desoxycorticosterone acetate supported the body weight significantly (all differences are more than 3 times the standard error of the means); but a dose of 5 mg. (plus pellet) to a single group of birds gave a less favorable result (table 1). Groups injected with 2 or 3 mg. of desoxycorticosterone acetate lost weight at first and thereafter slowly regained it; with lower dosage the loss was continuous (fig. 1).

In testing the effect of simultaneous dosage with both desoxycorticosterone acetate and prolactin the daily dose of the latter was uniformly restricted to 1 U of preparation number 680; since this preparation contained 11 Riddle-Bates units (15 I.U.) of prolactin per mg. a total of only 10/11 of a mg. was received by each bird during the 10-day period. One U daily of this prolactin reduced the amount of weight lost to less than half that lost by untreated hypophysectomized pigeons (fig. 2; table 1); this result confirms earlier tests (Schooley, Riddle and Bates, 1941) made on 9 birds with a daily dosage of 0.7 U of this same preparation of prolactin. A 1 mg. dose of desoxycorticosterone acetate decreased the loss from 21 to 10 per cent of the initial weight. When these same quantities of the two hormones were injected together all of the weight lost immediately after operation was regained and at killing the birds

TABLE 1. EFFECT OF CORTICAL AND PITUITARY HORMONES AND OF THYROXIN UPON BODY WEIGHT, FOOD AND WATER INTAKE IN YOUNG CARNEAU PIGEONS DURING 10 DAYS FOLLOWING HYPOPHYSECTOMY (AND CORRESPONDING 10 DAYS IN NORMALS)

EXP. NO.	TREATMENT	DAILY DOSE MG.	NO. OF BIRDS	INTAKE		BODY WEIGHT	
				FOOD PER DAY GM.	WATER PER DAY CC.	AT START OF TEST GM.	RESULT IN PER CENT OF INITIAL WEIGHT % σM
			Normal				
1	Untreated	—	70	35*	51*	473*	104.0*±0.87
2	Untreated	—	15	31	56	440	105.4±1.13
			Hypophysectomized				
3	Untreated	—	43	5	56	463	79.3±0.91
4	Desoxycorticosterone acetate (DCA)	P[1]	9	5	70	478	83.8±1.84
5		1	8	13	105	434	89.9±2.27
6		1+P[1]	10	16	120	467	90.5±2.67
7		2+P[2]	9	20	97	457	93.5±1.61
8		3	5	16	177	460	94.1±2.87
9		5+P	5	22	223	508	86.1±3.06
10	Adrenal cortex extract	0.1 cc.	4	13	120	501	85.9±1.35
11		0.2 cc.	5	17	217	519	86.6±1.96
12	Prolactin 680	1 U	9	10	59	434	92.1±2.96
13	Prolac. 680+DCA	1 U+1	20	23	103	446	100.8±1.29
14	Prolac. 680+DCA +Thyroxin	1U+1 5 μg.	14	21	130	454	97.7±2.21
15	Prolac. 680+DCA +Thyroxin	1 U+1 10 μg.	10	22	101	462	97.3±2.27
16	Thyroxin	10 μg.	4	5	44	445	81.6±2.48
17	Thyroxin	1 μg.	8	8	53	498	87.9±2.30
18	No. 549 FSH+	0.1	5	13	33	426	92.5±2.22

* Values found in a group of 33 birds (Schooley, Bates and Riddle, 1941).

[1] In addition, half of these pigeons were treated with equivalent amounts of testosterone propionate with no apparent effect on organ weights or food consumption. P = pellet.

[2] In addition, 5 of these pigeons were given 10 mg. of calcium pantothenate daily, with no apparent effect on organ weights or food consumption.

weighed 101 per cent of their initial weight (fig. 2; table 1). Individual group averages of the four separate tests were 100, 102, 103 and 99 per cent, respectively. The last group was chilled accidentally on the fifth night of the test, and a resulting loss of weight was only partially regained at the end of the test. Omitting this exceptional group the average of the other 3 is 102 per cent of the initial body weight. If the effects of prolactin and desoxycorticosterone acetate are additive, the expected final weight would be 103 per cent of the initial weight. Miller and Riddle (1943) showed that prolactin increases food intake and body weight in completely adrenalectomized pigeons.

Four pigeons injected with 10 μg. of thyroxin lost less weight than the average for all controls but more than the particular group of hypophysectomized controls that was tested simultaneously. In a single group of 8 pigeons

TABLE 1. (*continued*). EFFECTS OF CORTICAL AND PITUITARY HORMONES AND OF THYROXIN UPON ORGAN WEIGHT AND INTESTINE LENGTH IN YOUNG CARNEAU PIGEONS DURING 10 DAYS FOLLOWING HYPOPHYSECTOMY (AND CORRESPONDING 10 DAYS IN NORMALS)

EXP. NO.	INTESTINE		PANCREAS	LIVER	ADRENALS	THYROIDS	TESTES	CROP SACS
	LENGTH	WEIGHT (EMPTY)						
	CM.	GM. σM	GM. σM	GM. σM	MG.	MG.	MG.	GM.
				Normal				
1	107	12.4±0.32	1.47±0.03	11.2±0.25	33.3	84	44	1.29
2	109	12.1±0.53	1.59±0.05	9.5±0.35	29.0	73	35	1.19
				Hypophysectomized				
3	91	6.7±0.31	0.55±0.04	6.2±0.30	24.4	28	18	1.01
4	92	7.9±0.75	0.56±0.05	10.1±1.65	18.5	24	19	1.32
5	101	8.1±0.62	0.82±0.12	8.7±0.58	19.6	22	15	0.94
6	124	12.8±1.09	0.99±0.12	10.5±1.37	19.1	25	26	1.39
7	123	12.0±0.74	1.03±0.06	8.8±0.71	19.7	22	17	1.02
8	124	11.4±0.85	0.99±0.03	10.8±1.47	19.5	29	21	1.08
9	129	13.9±1.63	1.03±0.18	11.3±0.69	20.9	34	18	1.01
10	100	9.5±1.26	0.99±0.20	9.4±1.16	22.8	25	33	1.00
11	112	11.2±0.66	1.02±0.07	12.6±1.75	24.4	30	22	1.10
12	103	8.4±0.97	0.93±0.14	9.6±0.90	21.7	24	20	1.28
13	118	11.2±0.40	1.09±0.04	12.3±0.66	20.9	29	22	1.23
14	117	13.3±0.98	1.43±0.08	9.5±0.45	19.3	28	25	1.33
15	122	13.7±1.67	1.35±0.13	10.0±0.71	20.7	38	27	1.33
16	80	4.8±1.45	0.54±0.07	6.9±0.32	17.2	34	18	1.00
17	89	7.2±0.66	0.91±0.10	9.3±1.05	22.2	51	19	1.10
18	92	7.6±0.26	0.94±0.03	7.2±0.70	21.7	59	47	1.03

injected daily with 1 μg. of thyroxin the body weight seems to have been significantly increased (difference of 3 times the standard error) over both of the above-mentioned types of controls (table 1). The addition of 5 or 10 μg. of thyroxin to 1 U of prolactin and 1 mg. of desoxycorticosterone acetate did not result in an additional gain in weight but in a loss of 2 per cent. Injections of thyroxin were started on the third day of the experiment in these two tests. Testosterone propionate, when given together with desoxycorticosterone acetate, had no apparent effect on body weight (see footnotes, table 1). Small doses of protamine zinc insulin (0.3 U and 1 U per day; untabulated tests) increased the loss of body weight in two groups of hypophysectomized pigeons. Although there is good reason to suspect that nitrogen retention and support of body weight would accompany the combined use of insulin and prolactin such tests in pigeons have not been made, and indeed the tests will be without value until a satisfactory method of insulin administration is developed.

Food and water consumption. Food intake is markedly decreased by hypo-

physectomy, but this decrease was partially prevented by the injection of either adrenal cortex extract, desoxycorticosterone acetate or by 1 unit of prolactin. Although the partial maintenance of body weight was accompanied by partial maintenance of food intake there was no constant relation between the two (table 1). Thus with adrenal cortex extract food intake was sustained better than body weight and the same was true with 5 mg. of desoxycorti-

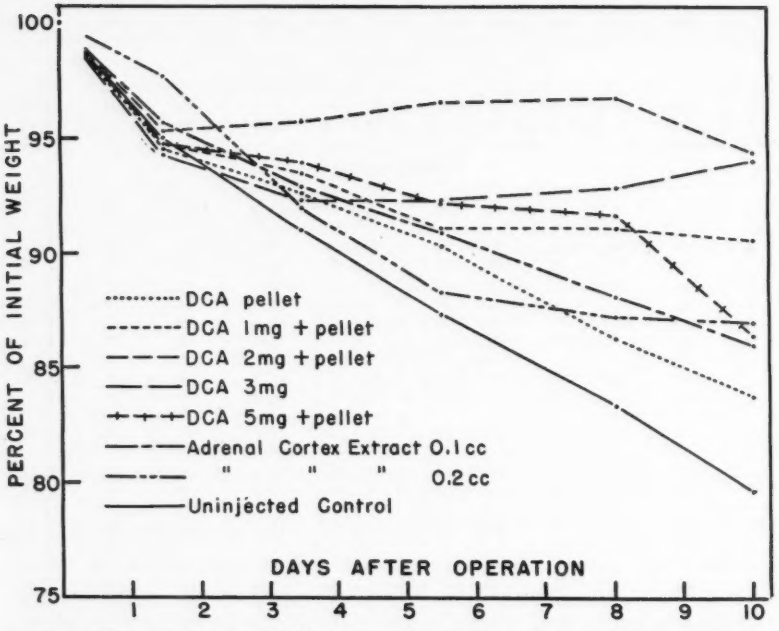


Fig. 1. Changes in body weight of hypophysectomized pigeons treated with desoxycorticosterone acetate and with adrenal cortex extract (Wilson). Untreated group, and those given lowest dosage, continue to lose weight throughout the 10-day period.

costerone acetate; the pigeons ate more food but lost more weight than did birds injected with smaller amounts of this steroid. The groups injected with both desoxycorticosterone acetate and prolactin ate about one-third less than do normal animals, but the pre-operation body weights of these birds was maintained and even slightly increased.

Water consumption was not measurably changed by hypophysectomy or by the daily injection of 1 U of prolactin. Cortical substances notably increased water consumption; the birds which received the highest dosage of the cortical hormones drank four times the normal amount of water (table 1). Since hydration of tissues might therefore share in the observed support of body weight the water content of the liver and breast muscle of 3 individuals in each of the groups listed below was measured. The amount of water, in percentage, is given first for the liver and second for the muscle in the following list: normal control, 71.9, 72.7; hypophysectomized control, 74.0, 71.8;

injected with desoxycorticosterone acetate and prolactin, 74.3; 72.6; injected with desoxycorticosterone acetate, prolactin and thyroxin, 75.4, 71.9. These data make it seem quite improbable that the injected materials sustained body weight through hydration of the tissues. The determinations of Schooley, Riddle and Bates (1941), like those noted here, show that the relative amount of water in the liver was slightly increased following hypophysectomy and also that prolactin did not induce a significant change in the water or ash

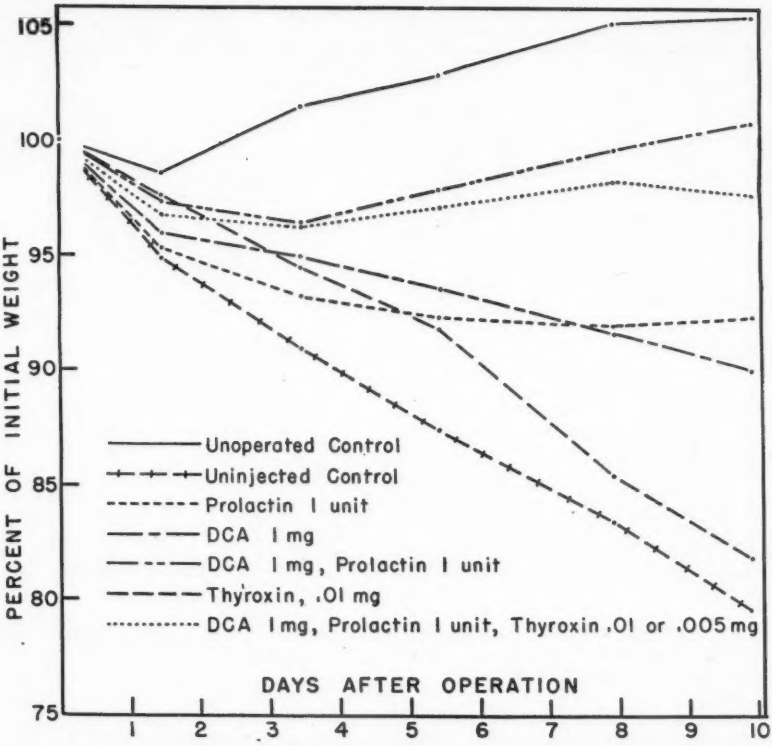


Fig. 2. Changes in body weight of normal untreated (top) and hypophysectomized pigeons injected with desoxycorticosterone acetate, thyroxin and prolactin.

content of the liver and breast muscle of hypophysectomized pigeons. Cortical hormones in amounts similar to those used here either failed to increase the water intake of adrenalectomized pigeons or increased it to no more than 77 cc. daily (Miller and Riddle, 1943).

Organ weights. The weights of all of the visceral organs and endocrine glands recorded in figure 3 and table 1 decreased following the removal of the pituitary. The weight of the adrenals and thyroids was not sustained by cortical hormones, prolactin or thyroxin. The adrenals of pigeons receiving desoxycorticosterone acetate were consistently smaller than those of hypophysectomized controls. In view of the variability of testis weights and the

small number of males the weight of the testes seems to have been maintained only by the preparation of gonadotropin, number 539. The weight of the crop sacs was consistently maintained at the unoperated level on all animals injected with 1 U of prolactin but cortical hormones had no clear effect. No observations were made concerning a persistence of effects of hormone administration after injections were stopped. Such an effect would not be expected since we are here dealing with a replacement therapy.

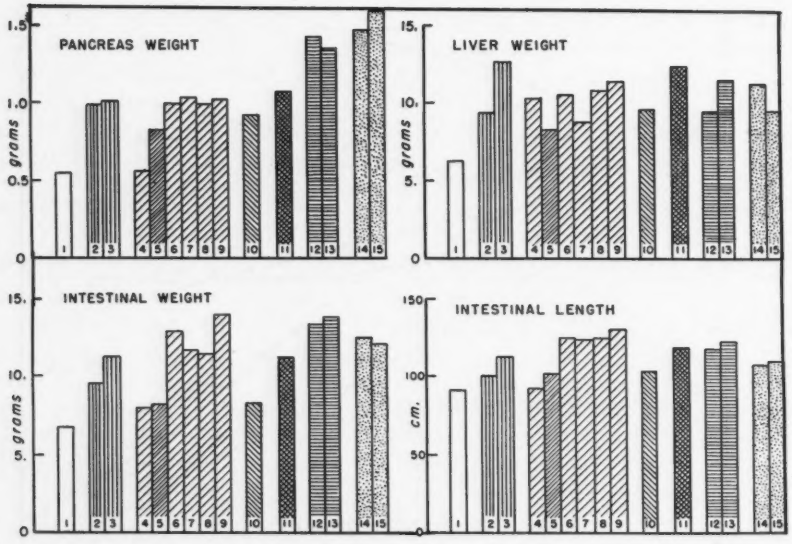


Fig. 3. Effects of 10 daily injections of adrenal cortical substance, prolactin and thyroxin upon maintenance of visceral tissues of hypophysectomized pigeons aged 1.5 months.

1. Hypophysectomized control (uninjected).
2. Adrenal cortex extract, 0.1 cc.
3. Adrenal cortex extract, 0.2 cc.
4. Desoxycort. ac. (DCA) pellet (P).
5. DCA, 1 mg.
6. DCA, 1 mg. +P.
7. DCA, 2 mg. +P.
8. DCA, 3 mg.
9. DCA, 5 mg. +P.
10. Prolactin 680, 1 U.
11. Prolactin 680, 1 U+DCA, 1 mg.
12. Prolactin 680, 1 U+DCA, 1 mg.+thyroxin 5 μg.
13. Prolactin 680, 1 U+DCA, 1 mg.+thyroxin 10 μg.
14, 15. Normal control, uninjected.

Although cortical hormones did not maintain the weights of the above glands they did sustain in varying degree the visceral organs of hypophysectomized pigeons (table 1, fig. 3). Livers and intestines of hypophysectomized pigeons receiving more than 1 mg. of desoxycorticosterone acetate were approximately equal in weight to those of unoperated controls, and the intestines (measured from gizzard to anus) were longer than normal; the weight of the pancreas, however, was only partially maintained. An implanted pellet plus 1 mg. of desoxycorticosterone acetate injected daily maintained the

weight of the liver, intestine and pancreas to almost the same degree as did larger amounts of this substance.

Except for the liver, the weight of which was maintained by 1 U of prolactin (table 1), either 1 mg. of desoxycorticosterone acetate or 1 U of prolactin only partially maintained the weight of the viscera. When these amounts of the two hormones were injected together the treated intestines became longer, although slightly lighter, than those of normal unoperated birds; the liver and pancreas of birds thus treated were significantly heavier than when either hormone was given alone. The partial maintenance of weight afforded by each of the two hormones appeared to be additive when they were given together. The values for the weight of the liver and intestine were slightly more, and for the pancreas only slightly less, than the sum of the separate gains.

The addition of 5 or 10 μg. of thyroxin to 1 mg. of desoxycorticosterone acetate and 1 U of prolactin resulted in further apparent increases in the weight of the intestine and pancreas but not in the liver. Statistically the increment in intestinal weight is not significant, although the mean weight obtained is higher; the increment in weight of pancreas from treatment with 5 μg. of thyroxin is 4 times, and that from 10 μg. of thyroxin is 2 times, the standard error. This action of thyroxin on pancreas and intestine is particularly notable since it was accompanied by no increase in food intake, and since 10 μg. of thyroxin alone showed no ability whatever to maintain the weight of these organs in hypophysectomized pigeons (table 1). Dosage with 1 μg. of thyroxin also probably failed to maintain the weight of these organs significantly if the large body size of this group of birds is given proper consideration. Schooley, Riddle and Bates (1941) found that thyrotropin partially sustained the weight of the pancreas, and that maintenance and overgrowth of the pancreas resulted when such preparations were combined with prolactin. In that investigation, and again in the present study, a preparation (number 549) containing large amounts of gonadotropin, thryrotropin and (some) corticotropin was tested at a dosage level of 0.1 mg. daily. This preparation at this low dosage level again sustained the weight of the pancreas, thyroid and testis (table 1). The loss of body weight by these 5 birds was somewhat less than it was in the earlier more extensive tests. Preparation 549 contained significant amounts of corticotropin as shown by its assay (see above) and by its effect, in higher daily dosage (3.1 mg.), on the adrenals of hypophysectomized pigeons (Schooley, Riddle and Bates, 1941). In view of the results produced by the cortical hormones in the present study, it is possible that this preparation exerted part or all of its favorable action through the adrenals.

DISCUSSION

The present data show that adrenal cortical hormones, like prolactin, although less effectively, can reduce or prevent the loss in weight of body and viscera which otherwise occurs during the 10 days immediately following hypophysectomy. Other investigators have found that desoxycorticosterone acetate stimulates an increase in the body weight of normal, adrenalectomized

and hypophysectomized rats (Wells and Kendall, 1940, a; Kendall, 1940; Fraenkel-Conrat, Simpson and Evans, 1943). The crystalline steroids from the adrenal which have an oxygen on C 11 were less potent or wholly unable to support growth in adrenalectomized rats (Kendall, 1940; Ingle, 1942) and may even cause a loss of weight in normal (Wells and Kendall, 1940, a, b; Ingle, 1941) and in hypophysectomized rats (Fraenkel-Conrat, Simpson and Evans, 1943). In other investigations adrenal cortical extracts did not sustain body weight in hypophysectomized rats (Atwell, 1932; Evans, Pencharz, Meyer and Simpson, 1933; Schumaker and Firor, 1934); normal unoperated rats so treated grew more rapidly in some instances (Corey and Britton, 1931; Fitzhugh, 1937) but not in another (Howard and Grollman, 1934). Thyroxin alone caused slight gains in the weight of hypophysectomized rats (Evans, Simpson and Pencharz, 1939) and temporarily accelerated growth in mice (Koger, Hurst and Turner, 1942) but this hormone has shown no definitely favorable effect on body weight in hypophysectomized pigeons. Smith (1933) and Evans, Simpson and Pencharz (1939) reported that thyroid substance synergized the growth activity of pituitary extracts in thyroidectomized-hypophysectomized rats. More recently Marx, Simpson and Evans (1942) stated that purified thyrotropic hormone also synergized this action of their purified growth hormone in hypophysectomized rats. This item provides welcome support to the view long maintained by Riddle and associates concerning the plural nature of the pituitary stimulus to bodily growth.

Still other evidence suggests that adrenal cortical hormone(s) participates with prolactin in the growth of normal pigeons. Unoperated Carneau pigeons grow more rapidly at 0.7 months after hatching and then have heavier crop sacs (Bates, Riddle, Lahr and Schooley, 1937) than the older pigeons used here; those heavier crop sacs apparently indicate a greater output of prolactin. The adrenals likewise were heavier and, according to cytological criteria, they were secreting more actively at this younger age than later (Miller and Riddle, 1942, a).

It was earlier shown (Schooley, Riddle and Bates, 1941) that the pituitary sustains the weight of the visceral organs of pigeons in large measure by maintaining appetite and food consumption. Liver weight was maintained fully in hypophysectomized pigeons by force-feeding alone. The weight of the pancreas, however, seems to be sustained in part by an action of the pituitary other than its action on food consumption. The fact that higher doses of desoxycorticosterone acetate are now observed to sustain intestinal weight better than did force-feeding in the above-mentioned study provides evidence that this steroid, like prolactin, has a special action on the intestine.

Desoxycorticosterone acetate when given either alone or with prolactin, like force-feeding or prolactin in the earlier work (Schooley, Riddle and Bates, 1941) partially sustained the weight of the pancreas; but it is notable that none of these treatments actually maintained the weight of that organ. In view of these facts we may attach importance to the new observation that with the addition of thyroxin to desoxycorticosterone acetate and prolactin

the weight of the pancreas is almost fully maintained. Similarly, the use of thyroxin in this combination has suggested but not proved its ability to increase intestinal weight. These results are especially notable since 1 and 10 μg. of thyroxin given alone did not significantly sustain the weight of either the intestine or the pancreas. These effects of thyroxin both confirm and extend the earlier conclusion (Schooley, Riddle and Bates, 1941) that thyrotropin enhances the action of prolactin upon the pancreas and intestine.

The present study has shown that derivatives of both adrenals and thyroids supplement the direct sustaining action of a primary pituitary product, prolactin, upon the intestine and pancreas; likewise certain adrenal derivatives partially sustain body weight following hypophysectomy. The production of these adrenal hormones seems to be regulated, in part at least, by the pituitary hormone, corticotropin. Our results thus indicate that in pigeons more than one pituitary hormone promotes bodily growth, and this fact has bearing on the general question of the number and nature of the pituitary hormones which influence somatic growth.

SUMMARY

White Carneau pigeons, 1.5 months old, were hypophysectomized and injected daily for 10 days with adrenal cortical hormones, thyroxin and prolactin. These substances were administered separately and in various combinations.

Adrenal cortex extract and desoxycorticosterone acetate partially prevented the 21 per cent loss of body weight which otherwise occurs during 10 days following removal of the anterior pituitary. Optimal effects of desoxycorticosterone acetate were obtained with daily doses of 2 or 3 mg. Although either 1 mg. of desoxycorticosterone acetate or 1 U of prolactin only partially sustained body weight these quantities, when injected together, had additive effects; together they maintained or more than maintained the pre-operative weight of pigeons. Five or 10 μg. of thyroxin added to this combination did not induce additional gains in body weight.

Following hypophysectomy cortical hormones partially sustained the food consumption and stimulated water intake, but there was no significant hydration of breast muscles or livers.

Adequate doses of cortical hormones maintained the weights of the intestine and liver, and partially sustained the weight of the pancreas. Hypophysectomized pigeons given 1 mg. of desoxycorticosterone acetate and 1 U of prolactin had livers heavier and intestines longer, although somewhat lighter, than those of unoperated pigeons; the pancreas retained about 70 per cent of the normal weight, and these were thus nearly twice as heavy as those of untreated controls. When 5 or 10 μg. of thyroxin was added to the above amounts of prolactin and desoxycorticosterone acetate the weight of the intestine was probably increased and that of the pancreas was increased by 20 per cent; these changes occurred without increase in food consumption. Since force-feeding alone in an earlier study only partially maintained the

weight of the intestine and pancreas it is concluded that, like prolactin, desoxycorticosterone acetate and thyroxin have a specific action upon these organs.

REFERENCES

ATWELL, W. J.: *Endocrinology* 16: 639. 1932.
BATES, R. W., O. RIDDLE, E. L. LAHR AND J. P. SCHOOLEY: *Am. J. Physiol.* 119: 603. 1937.
BATES, R. W., O. RIDDLE AND R. A. MILLER: *Endocrinology* 27: 781. 1940.
COREY, E. L., AND S. W. BRITTON: *Am. J. Physiol.* 99: 33. 1931.
EVANS, H. M., R. I. PENCHARZ, K. MEYER AND M. E. SIMPSON: Mem. Univ. Calif. 11: 339. 1933.
EVANS, H. M., M. E. SIMPSON AND R. I. PENCHARZ: *Endocrinology* 25: 175. 1939.
FITZHUGH, O. G.: *Am. J. Physiol.* 118: 677. 1937.
FRAENKEL-CONRAT, H., M. E. SIMPSON AND H. M. EVANS: *J. Biol. Chem.* 147: 99. 1943.
HOWARD, E., AND A. GROLLMAN: *Am. J. Physiol.* 107: 480. 1934.
INGLE, D. J.: *Endocrinology* 29: 649. 1941.
INGLE, D. J.: *Endocrinology* 31: 419. 1942.
KENDALL, E. C.: *Proc. Staff Meet. Mayo Clinic* 15: 297. 1940.
KOGER, M., V. HURST AND C. W. TURNER: *Endocrinology* 31: 237. 1942.
MARX, W., M. E. SIMPSON AND H. M. EVANS: *Proc. Soc. Exper. Biol. & Med.* 49: 594. 1942.
MILLER, R. A., AND O. RIDDLE: *Am. J. Anat.* 71: 311. 1942, a.
MILLER, R. A., AND O. RIDDLE: *Anat. Rec.* (Suppl.) 84: 40. 1942, b.
MILLER, R. A., AND O. RIDDLE: *Proc. Soc. Exper. Biol. & Med.* 52: 231. 1943.
SCHOOLEY, J. P., O. RIDDLE AND R. W. BATES: *Am. J. Anat.* 69: 123. 1941.
SCHUMAKER, H. B., AND W. M. FIROR: *Endocrinology* 18: 676. 1934.
SMITH, P. E.: *Proc. Soc. Exper. Biol. & Med.* 30: 1252. 1933.
WELLS, B. B., AND E. C. KENDALL: *Proc. Staff Meet. Mayo Clinic* 15: 133. 1940, a.
WELLS, B. B., AND E. C. KENDALL: *Proc. Staff Meet. Mayo Clinic* 15: 324. 1940, b.

PITUITARY-DIABETES IN THE CAT: RECOVERY FOLLOWING PHLORHIZIN TREATMENT[1]

F. D. W. LUKENS, F. C. DOHAN AND M. W. WOLCOTT
From the George S. Cox Medical Research Institute, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA

THE PRODUCTION of pituitary-diabetes in the cat, the course of the lesions in the islands and the recovery of animals given early treatment with insulin have been previously reported (Lukens and Dohan, 1942). In discussing these results it was noted that, in experimental diabetes, damage to the islands of Langerhans was always associated with hyperglycemia. In like manner the restoration of the early lesion of hydropic degeneration (Copp and Barclay, 1923; Lukens and Dohan, 1942) followed the reduction of hyperglycemia to normal levels. In the case of insulin treatment, this recovery of the islands might have been due to some effect of insulin other than the lowering of blood sugar. Preliminary studies on the treatment of such diabetic animals with phlorhizin were cited to emphasize the possible importance of the level of blood glucose *per se*.

The present report describes the recovery of cats with pituitary-diabetes after treatment with phlorhizin early in the course of the disease. In addition we have included a few experiments which help to define the metabolic conditions under which phlorhizin exerts its beneficial influence on the diabetes. The differences between the effects of phlorhizin and insulin permit a provisional estimation of the factors involved in the recovery of this type of experimental diabetes.

Minkowski (1893) first gave phlorhizin to depancreatized dogs and found that it produced a fall in the blood sugar, a finding which Hedon (1897) confirmed. Allen (1913, 1923) cited the early observations on the islands in phlorhizinized animals and used the drug in his own studies. He gave phlorhizin to 3 partially depancreatized non-diabetic dogs for 45, 70 and 95 days. At the end of this treatment normal islands were found at biopsy and there was the usual disappearance of the glycosuria. We have confirmed these findings in 4 cats treated with phlorhizin for a month. Allen (1923) also used phlorhizin in partially depancreatized dogs in an effort to determine the part played by the blood sugar in the development of island lesions. His results were inconclusive because of the spontaneous fluctuations in the diabetes of his animals. Recent studies by Mirsky, Heiman and Broh-Kahn (1937) and by Houssay and Foglia (1937) have also demonstrated the power of phlorhizin to lower the blood sugar in diabetes.

The early work on the action of phlorhizin has been reviewed by Nash

Received for publication March 24, 1943.

[1] A preliminary report of certain of these experiments was presented before the American Physiological Society in 1941. *Am. J. Physiol.* 133: P368, 1941.

(1927). Although this drug may affect several organs, its major action is to interfere with the re-absorption of glucose by the renal tubules. This concept has been strengthened by the recent study of Beck (1942).

METHODS

The procedures previously used in the production and study of pituitary-diabetes in the cat have been employed (Lukens and Dohan, 1942).

Phlorhizin (Eimer and Amend) was suspended in sesame or olive oil, usually the latter. It was given subcutaneously in doses of 0.1 to 0.4 gm. per day, the usual dose being 0.2 gm. daily. This is less than the amount traditionally employed to cause complete phlorhizin glycosuria. After daily injections of phlorhizin in oil had been given for 2 to 3 weeks, the glycocuria disappeared 3 to 15 days after the last injection. This variability has been attributed to the difference in the rate at which the oil depots were absorbed. Aseptic technique was not used and there were no local infections from this treatment. Phlorhizin has usually been given to fasting normal animals in large dosage. Its use in fed, diabetic animals in small dosage has been success-

TABLE 1. ESTABLISHED DIABETES IN CATS RECOVERING AFTER PHLORHIZIN TREATMENT

CAT NO.	GLYCOSURIA		DIETARY GLUCOSE EXCRETED[2]	DURATION OF PHLORHIZIN TREATMENT	DURATION[3] OF RECOVERY	CHANGE IN WEIGHT IN FIRST 2 WEEKS	
	DURING[1] INJECTION OF APE	INTERVAL BETWEEN APE AND HLORHIZIN				DURING PHLORHIZIN	AFTER PHLORHIZIN
	days	*days*	%	*days*	*days*	*gm.*	*gm.*
S23	33 **2–34**	36 **35–70**	11	12 **71–82**	31 **89–119**	−300	+100
S14	13 **2–14**	17 **15–31**	25	14 **32–45**	22 **49–70**	+200	+550
S34	19 **3–21**	80 **22–101**	31	15 **102–116**	15 **133–147**	−150	+150
S6	17 **4–20**	18 **21–38**[4]	63	20 **39–58**	17 **66–82**	+100	+500
T8	20 **2–21**	10 **22–31**	77	20 **32–51**	110 **54–164**	0	+300
	9 **165–173**	11 **174–184**	92	20 **185–204**	24 **208–231**	−50	+350
T7	23 **2–24**	13 **24–37**	87	20 **38–57**	54 **61–114**	+50	+200
S19	25 **2–26**	17 **27–43**	94	21 **44–64**	35 **77–111**	+150	+350

[1] In this table the figures in boldface show the days from the first injection of anterior pituitary extract and permit comparison with days printed in boldface in the text and figures.

[2] The percentage of available glucose in the meat diet (D :N= 3.65) which was excreted in the urine has been used as an approximate index of the severity of the diabetes.

[3] Non-diabetic period from cessation of phlorhizin action to end of experiment (see text).

[4] Insulin given for 6 days before the biopsy made on the 36th day.

ful in controlling the blood-sugar level and has avoided the extreme metabolic disturbances seen in the fully phlorhizinized animal.

RESULTS

The original observations presented in the tables and figures will be briefly related in the text to our previous findings in pituitary-diabetes, which cannot be repeated at length. Tables 1 and 2 record the functional and anatomical recovery from established pituitary-diabetes which followed early treatment with phlorhizin. As diabetes was produced and treated a second time in cat T8, there were 8 recoveries in 7 animals. The duration of extract injection fell within the range of previous experiments and the brief period of extract needed to make cat T8 diabetic the second time conforms to our previous experience with the repeated production of diabetes (Lukens and

TABLE 2. BLOOD SUGAR AND CONDITION OF ISLANDS OF CATS SHOWN IN TABLE 1

CAT NO.	BLOOD GLUCOSE			PANCREATIC ISLANDS	
	BEFORE[1] TREATMENT	AVERAGE DURING TREATMENT	AVERAGE AFTER TREATMENT	BEFORE TREATMENT	AFTER TREATMENT
	mg./100 cc.	mg./100 cc.	mg./100 cc.		
S23	133, 130	79		Hydropic ++	Normal or slight degranulation of beta cells.
S14	154, 126	70	85		
S34	219, 196	87	104		
S6	299, 268	83	79	Hydropic +++	
T8	287, 336	75	88		
	357, 326	78	89		
T7	292, 294	78	89		
S19	322, 276	78	87		

[1] The last two determinations before treatment with phlorhizin are presented.

Dohan, 1942). The criteria of persistent diabetes and of functional recovery, which have been previously described, were fulfilled by all of these animals. As in our studies with insulin, recovery meant *a*) the absence of glycosuria; *b*) the normal level of blood glucose, and *c*) the capacity to gain weight; all of these *d*) on the same adequate diet eaten prior to treatment. The pathological changes of the islands during diabetes and the occasional minor changes after recovery follow the description already given (Lukens and Dohan, 1942). The significance of this type of recovery will be re-appraised in the discussion. Cat S6 was treated with insulin for 6 days (**29–35**) and the biopsy made on the **36th** day showed marked hydropic degeneration (fig. 2A). From previous experience we assume that this brief use of insulin played no part in the subsequent recovery. The animals have been listed in the order

of the severity of the diabetes, which has varied widely. The duration of phlorhizin treatment ranged from 12 to 21 days; the dose was 0.2 gm. daily in olive oil, except in cat S6 which received 0.4 gm. After the last dose of phlorhizin 3 to 17 days were required for the effect of the drug to disappear, a variation attributed to differences in the rate at which the oil injections were absorbed. Because these days comprise a less exact portion of the treatment period, they have been omitted from the table, as shown by the interval between the last day of treatment and the first day of the recovery period. The duration of recovery has been satisfactory for these acute experiments. Three animals had oral sugar tolerance tests in the recovery period. After 1.8 gm. glucose per kg. the highest blood sugar in S34 and S6 was 118 mg. per 100 cc. in the 2-hour test. When cat T8 was tested on the **230th** day, the blood sugar rose to 177 at 2 hours, but no glycosuria occurred. During treatment a loss of weight was observed in 3 and a gain in weight in the animals in 4 of the experiments. The fact that some animals gained weight during phlorhizin treatment may be cited as evidence that undernutrition *per se* was not the factor responsible for the improvement in these animals. This does not nullify the value of undernutrition in other circumstances. The consistent gain in weight after treatment would be expected when phlorhizin glycosuria had ceased and all of the food was assimilated with adequate function of the islands of Langerhans. In the experiments on cat T8 a biopsy of the pancreas taken on the **115th** day showed normal islands. After operation the animal had 14 days of glycosuria, which was controlled for the subsequent 17 days by reducing the diet to 100 gm. of beef daily. The full diet of 200 gm. of beef was resumed for the remaining 12 days of this period and the animal maintained a normal blood sugar. Consequently, the whole period of 110 days has been used as the duration of recovery.

In table 2 the fasting blood-sugar levels and the condition of the islands of Langerhans have been summarized. The last two determinations of blood glucose prior to treatment have been selected, and in every case the second value was determined just before the first dose of phlorhizin. During and after treatment the average blood-sugar levels tabulated were based on 2 to 11 estimations per animal for each period and no values exceeded the normal range. In addition a number of postprandial blood sugars were found to be normal. Pancreatic biopsies were performed in 2 animals as noted. The presence of hydropic degeneration at this stage of pituitary-diabetes in the cat has been observed so regularly (Lukens and Dohan, 1942), that no more pre-treatment biopsies were deemed necessary. With the exception of S19 the pancreas was examined in each animal in the recovery period. At this time the islands were largely restored to normal appearance. Thus, cats S14, S6, and T8 (1st recovery) and T7 had islands of normal appearance; cats S23 and S34 had slight degranulation of the beta cells, and in the latter the islands were somewhat few and small. This suggestion of early atrophy of the islands in cat S34 is not surprising in view of the duration of the diabetes before treatment. At the autopsy of cat T8 the islands were few in number and some were small, indicating early atrophy. There were, however, many

large islands which appeared quite normal histologically by Bensley as well as by routine stains.

The diets used in these experiments are not tabulated because they were so nearly uniform. Cats S23 and S34 had 200 gm. of beef daily with very slight irregularities. Cat S14 had 200 gm. before phlorhizin and 250 gm. daily during and after treatment (fig. 1); in cat S6 this was reversed, 250 gm. being the diet before treatment and 200 gm. during and after. Cats T8, T7 and S19 ate 200 gm. of beef daily throughout all of the experiments.

Figure 1 illustrates the course of the production of diabetes, its treatment and recovery in cat S14, and figures 2A and B show the state of the islands of S6 before and after phlorhizin. In figure 1 the combination of phlorhizin and the increased diet (250 gm.) resulted in an increased glycosuria. This was true

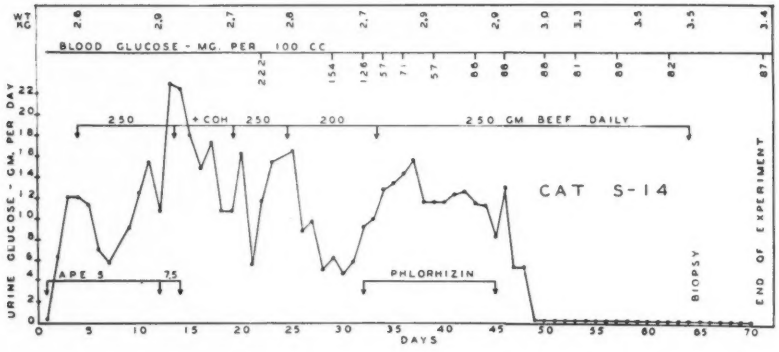


Fig. 1. Protocol of cat S14. (See tables 1 and 2.) Partial pancreatectomy was performed 7 days before the first day shown on the chart. The figures above the arrows which include days 1 to 14 indicate the cc. of anterior pituitary extract (APE) given daily during this period. The dose of phlorhizin was 0.2 gm. daily for the period indicated. After anterior pituitary extract was discontinued, the diminution in glycosuria was in part due to the successive reductions in diet. Where the diet is marked "+COH" the animal received 250 gm. of beef and 8 gm. of starch daily. When phlorhizin was given, the increased glycosuria was in part due to the increased diet.

of the other mildly diabetic cats on a constant diet. In contrast, it was found that when phlorhizin was given to severely diabetic animals the glycosuria diminished. Figure 3 shows the variations in glucose excretion during phlorhizin treatment and relates them to the severity of the preceding diabetes. The curves show that when the glycosuria was slight it was increased by the administration of phlorhizin. However, when the diabetes was severe (T8, S19) the glycosuria decreased in the first week of treatment until it reached the level caused by phlorhizin alone. The degree of glycosuria produced when this dose of phlorhizin was used with a diet of 200 gm. of beef is shown in the later part of the treatment periods in figure 3 and has been demonstrated by additional experiments in normal animals. The diminution in glucose excretion which occurred when phlorhizin was used in severe diabetes has been attributed to the immediate beneficial effect of the lowered blood-sugar level on the hydropic beta cells. The same prompt benefit to the islands of Langer-

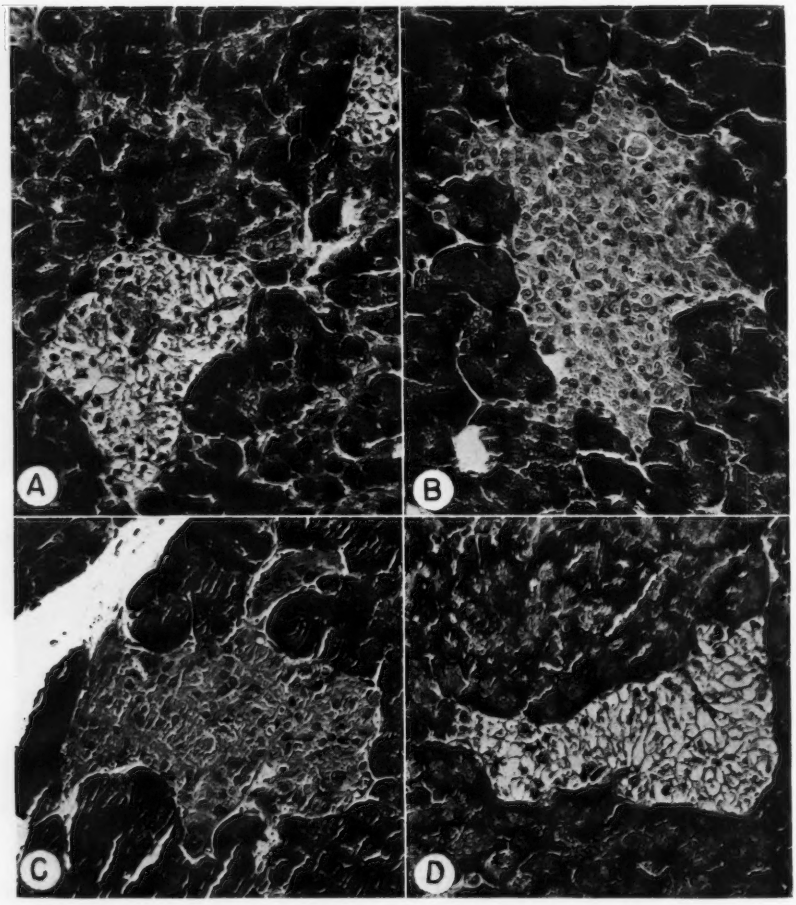


Fig. 2. A. Pancreatic island of cat S6 (×200) before phlorhizin on day 36. B. Island of cat S6 on day 82, 17 days after the end of the phlorhizin treatment (cf. tables 1 and 2). C. Island of Langerhans of cat T13 (×200) after 21 days of anterior pituitary extract with phlorhizin also given for the last 15 days of this period. D. Island of cat T13 after 21 days of anterior pituitary extract alone (cf. table 3 and fig. 4).

hans presumably occurred in mild diabetes but here it was obscured by the glycosuria due to the action of the drug on the kidney.

As in the case of insulin the response to treatment depended on the stage of the pathological process. In contrast to the results described above, no recovery followed the use of phlorhizin late in the disease when the damage to the islands had become irreversible. This was observed in one cat (T2) which had mild diabetes. On the 70th day of glycosuria a biopsy of the pancreas was performed and on the 82nd day phlorhizin was begun. After 14 days of treatment the drug was discontinued and in a week hyperglycemia

recurred and glycosuria of the degree found before treatment persisted. The biopsy made before treatment revealed marked atrophy of the pancreatic islands. A similar experience was encountered in a dog which had pituitary-diabetes of 3 years' duration. This dog, weighing 12 kg., was given 0.2 gm. of phlorhizin daily for 25 days during which the blood sugar ranged from 61

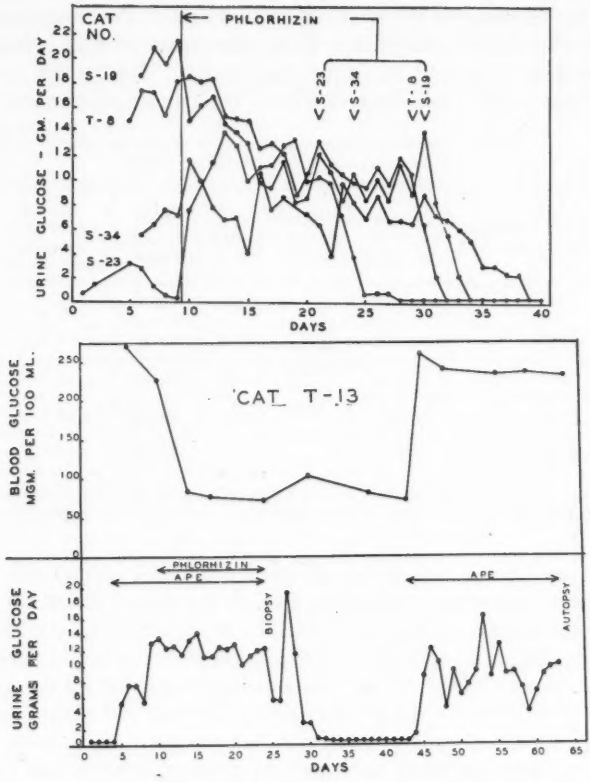


Fig. 3. Effect of early phlorhizin treatment on the glycosuria of cats with mild and severe diabetes. The chart has been arranged to compare the first days of treatment. The markers beneath the cat numbers indicate the end of treatment.

Fig. 4. Blood sugar and glucose excretion in cat T13 when anterior pituitary extract (APE) was given with and without phlorhizin. Partial pancreatectomy was done 13 days before the first day shown on the chart. Figure 2 shows the islands of Langerhans at biopsy and autopsy (cf. table 3).

to 100 mg. per cent. After the drug was withdrawn, glycosuria continued and hyperglycemia returned.

The prevention of pituitary-diabetes by phlorhizin. In the preceding experiments the blood sugar of diabetic animals was lowered after the activity of the previously injected pituitary extract had subsided. In order to learn whether phlorhizin would counteract the hyperglycemic action of anterior

pituitary extract itself, a few partially depancreatized cats were given anterior pituitary extract and phlorhizin simultaneously. During this treatment the blood sugar was found to be normal. After a rest period of 7 to 10 days, anterior pituitary extract alone was given to the same animal. The resulting hyperglycemia showed that the extract was potent and that the animal was not refractory. Figure 4 is the graphic protocol of one such experiment. In addition to determining the blood sugar, biopsies of the pancreas were performed at the end of each period. These demonstrated that phlorhizin protected the beta cells from hydropic degeneration. The results are summarized in tables 3 and 4, and figure 2, C and D illustrate anatomically the protection given to the islands by phlorhizin. In each animal the diet was constant throughout the experiment, so that the glucose excretion with and without phlorhizin could be compared. The average daily glycosuria was not significantly altered by the addition of phlorhizin and was the same on diets of 150 or 200 gm. of beef. There was a definite tendency for the qualitative ketonuria (nitroprusside test) to be increased when phlorhizin was added. In another series of 7 animals, thyroid biopsies were made when anterior pituitary extract was given with and without phlorhizin, and the results indicated that there was no inhibition of the thyrotropic effect.

TABLE 3. PREVENTION OF ISLAND DAMAGE BY PHLORHIZIN GIVEN DURING ANTERIOR PITUITARY EXTRACT (APE)

CAT NO.	PHLORHIZIN and APE	BLOOD GLUCOSE (RANGE)	ISLANDS AT END OF PERIOD	APE	BLOOD GLUCOSE (RANGE)	ISLANDS AFTER APE; HYDROPIC DEGENERATION
	days	*mg./100 cc.*		*days*	*mg./100 cc.*	
S12	12	69–110	normal or slight degranulation	13	117–251	++++
S28	16	75–112		15	160–297	+-
T13	15[1]	72–84		21	242–260	++++
T15	15[1]	65–66		21	158–323	++++

[1] Anterior pituitary extract was also given for 6 days before phlorhizin was begun so that the total period on pituitary extract was 21 days as in the subsequent experiment with anterior pituitary extract alone (cf. fig. 4).

Miscellaneous Observations

In addition to these experiments with pituitary-diabetes, phlorhizin has been given to 2 cats which were made diabetic by the removal of a larger proportion of the pancreas. It was possible to demonstrate the reduction of hyperglycemia and morphologically the restoration of the islands in these animals. No further studies of this sort were pursued as the results paralleled those obtained by Copp and Barclay (1923) who used insulin in this type of diabetes. Sustained functional recovery was not possible in such animals because the pancreatic remnants were originally too small to prevent diabetes.

A few acute experiments were conducted to observe the effect in normal and depancreatized cats of the dosage of phlorhizin (0.2 gm. per day) used in the preceding experiments. The results have been collected in table 5 in which the following points may be noted. In group B the glycosuria was less than that of fully phlorhizinized animals reported by the older workers, but the nitrogen excretion was significantly increased above that of fasting untreated cats (group A). The data in group C show the glucose and nitrogen excretion of a series of depancreatized cats; those for group D show that

TABLE 4. GLYCOSURIA AND KETONURIA DURING ANTERIOR PITUITARY EXTRACT (APE) INJECTION WITH AND WITHOUT SIMULTANEOUS PHLORHIZIN TREATMENT

CAT NO.	MEAT—BOTH PERIODS	AV. URINE GLUCOSE[1]		KETONURIA	
		APE AND PHLORHIZIN	APE	APE AND PHLORHIZIN	APE
	gm./day	gm./day	gm./day		
S12	200	11.6	11.6	+	0
S28	200	12.1	12.5	+++	trace
T13	150	12.3	10.6	0	0
T15	150	10.6	11.6	+++	0

[1] The average urine glucose has been calculated from the excretion for 6 consecutive days in the middle of each experimental period.

TABLE 5. RESPONSE OF FASTING NORMAL AND DEPANCREATIZED CATS TO PHLORHIZIN

NO. OF CATS	PERIOD	AVERAGE URINARY GLUCOSE	AVERAGE URINARY NITROGEN	D:N OF AVERAGES
	days	gm./kg./day	gm./kg./day	
		A. Normal cats, untreated		
9	5	0	0.63±0.04	—
		B. Normal cats, 0.2 gm. phlorhizin daily		
5	5	2.09±0.20	1.01±0.08	2.10
		C. Depancreatized cats, untreated		
21	3	2.93±0.17	1.24±0.06	2.36
		D. Depancreatized cats, 0.2 gm. phlorhizin daily		
7	2 to 3	3.60±0.30	1.29±0.09	2.78

In groups A and B the values of the last 4 days of the 5-day periods have been used. In groups C and D the days of operation and death have been excluded as in previous calculations (Lukens and Dohan, 1938)

when phlorhizin was given to depancreatized cats, the glucose excretion was slightly greater than it was after pancreatectomy alone. In groups C and D the variance has been computed from the pooled observations of 28 animals. This slightly increased glycosuria in group D is in accord with the observations of Soskin, Levine and Lehmann (1938) that less glucose is oxidized at low blood-sugar levels, but our figures lack statistical validity. The nitrogen excretion in group D was certainly no less than that of the controls (group C). Qualitative tests indicated a greater tendency to ketonuria in the phlorhizin-treated series. In the animals of series D, the blood sugar was determined daily and was normal except in a few animals at the time of death.

These occasional high terminal blood sugars have been attributed to the reduction of renal function due to premortal circulatory collapse. Lepine (1909), Houssay and Foglia (1937) and others have found that phlorhizin did not lower the blood sugar of diabetic dogs after chemical injury to the kidney or nephrectomy. In brief, the results in table 5 show that in both normal and depancreatized cats, the excretion of glucose and nitrogen and the qualitative tests for ketonuria tend to be diabetic in character. Phlorhizin certainly fails to inhibit these phenomena of diabetes and appears to be unlike insulin in this respect.

DISCUSSION

The criteria and limitations of the recovery from diabetes observed in these animals have already been discussed (Lukens and Dohan, 1942). We

TABLE 6. COMPARISON OF THE ACTION OF PHLORHIZIN AND INSULIN[1]

PHLORHIZIN	INSULIN
A. Similar Effects	
1. Reduction of Hyperglycemia[1]	
2. Restoration of Hydropic Islands[1]	
B. Different or Opposite Effects	
1. Impairs reabsorption of glucose by kidney-primary action	1. No such action on kidney is known
2. Increases protein catabolism (N excretion)[1]	2. Decreases protein catabolism (N excretion)
3. Increases ketogenesis[1]	3. Decreases ketogenesis
4. Amount of food assimilated by diabetic cats may be increased, *decreased* or unchanged	4. Food assimilated by diabetics is increased[1]
5. Slight gain or loss of weight occurs[1]	5. Marked gain in weight is usual[1]
C. Uncertain Effects, Common Assumptions Listed	
The following effects are probably secondary to loss of glucose:	The following may include the primary action of insulin:
1. Decreases COH oxidation (secondarily)	1. Increases COH oxidation
2. Liver and muscle glycogen is usually decreased but may be increased by added COH or insulin	2. Increases liver and muscle glycogen in the diabetic

[1] Observed or confirmed in the course of the experiments reported or cited herein.

wish to emphasize the point that the recovery of the animals in these experiments is not an achievement claimed, but a process described. The results indicate the degree or extent to which the islands of Langerhans may improve under certain circumstances. From this point of view the findings in animals simulate in many ways the remission or improvement occasionally seen in diabetes mellitus in man.

As phlorhizin and insulin may each bring about the recovery of pituitary-diabetes in the cat a comparison of the actions of these drugs is in order. To compare them, table 6 has been prepared as an outline for discussion. Such a summary is not designed to portray an exact picture of two substances whose actions are far from fully understood, for the comparison in table 6 refers chiefly to their effects in pituitary-diabetes. There have been two ob-

vious similarities between phlorhizin and insulin in our diabetic animals. Each lowered the level of blood sugar and each one led to the recovery of hydropic degeneration of the islands of Langerhans. Except for these features, the tables presents only contrasts between insulin and phlorhizin. Of these contrasts some have been mentioned in the results presented herein or previously, whereas others have been described in the literature. Some items have been well established, others are admittedly uncertain. They are presented with the following comments.

1. The reduction of hyperglycemia. It is agreed that this is the result of the effect of phlorhizin on the kidney. That it is not due to the stimulation of insulin secretion is indicated by the work of Houssay and Foglia (1937) who have reported a slightly diminished secretion of insulin during phlorhizin. This may be compared with the findings cited by Nash (1927) that the insulin content of the pancreas is normal in phlorhizinized animals. In contrast, a distinct decrease in insulin content occurs when insulin is administered (Haist and Best, 1940).

2. The action of phlorhizin is on the kidney. This represents the generally accepted conclusion. The possible influence of phlorhizin on other organs is beyond the scope of this discussion (Nash, 1927; Beck, 1942; Soskin, Levin and Lehmann, 1938).

3. "Increases protein catabolism." Our experiments have confirmed the increased nitrogen excretion of normal cats and have indicated that the increased urinary nitrogen of depancreatized cats is maintained under treatment with phlorhizin. In contrast to this is placed the sparing action of insulin on protein metabolism as measured by nitrogen excretion. No attempt was made to measure the nitrogen excretion during treatment of the meat-fed cats with pituitary-diabetes as the changes in endogenous N metabolism are usually obscured by such a diet.

4. The antiketogenic action of insulin has been contrasted with the frequent appearance of ketonuria in the phlorhizin experiments. Most writers (Nash, 1927; Soskin, *et al.*, 1938; Deuel, Wilson and Milhorat, 1927) regard this ketogenic effect of phlorhizin as secondary to its action on the kidney; *i.e.* secondary to the loss of glucose or to excessive gluconeogenesis. Table 6 is not meant to imply that phlorhizin is primarily ketogenic; merely that it is far from being as antiketogenic as insulin.

5. Fat formation, measured as gain in weight (cf. table 1) occurred to a limited degree in a few cats during phlorhizin treatment. Almost all animals except those severely diabetic kept a fairly constant weight during treatment. Most of the insulin-treated diabetic animals gained weight rapidly.

6. Common postulates about the oxidation of glucose are given (Nash, 1927; Soskin, *et al.*, 1938; Deuel, *et al.*, 1927). As in the case of phlorhizin ketonuria this is regarded as being secondary to the deficiency in the supply of glucose because the RQ of the phlorhizinized dog is raised by glucose administration (Deuel, Wilson and Milhorat, 1927).

7. It is agreed that muscle glycogen is increased by insulin and is usually lowered by phlorhizin (Nash, 1927) although it may be maintained during phlorhizin if the animal is well fed. The effect on liver glycogen is less clear. Phlorhizin lowers liver glycogen in normal animals (Nash, 1927) and insulin increases it in diabetic animals. The difference in circumstances makes the comparison highly approximate and the effects of phlorhizin on glycogen are generally regarded as secondary manifestations.

With due allowance for the controversial elements in table 6, the apparent contrast between insulin and phlorhizin, except in their effect on the diabetic blood-sugar level, affords a new type of evidence that the normal level of blood glucose is the factor promoting the recovery of the islands of Langerhans.

Finally, the facts to be considered in estimating the rôle of hyperglycemia in the pathogenesis of diabetes may be briefly reviewed. Neither partial pancreatectomy nor anterior pituitary extract cause island lesions in the absence of hyperglycemia. Partial pancreatectomy and anterior pituitary extract each lead to hydropic degeneration after hyperglycemia has been present for a week or two. While the lesions are still reversible (cat), the island damage, in the presence of hyperglycemia, progresses to irreversibility, but may not advance in severity. The elevation of the blood sugar by perfusion of glucose has resulted in early lesions of the islands. If treated early, the pancreatic islands are restored by various procedures; *viz.*, insulin, low diet, phlorhizin and adrenalectomy (one experiment), which return the blood sugar to normal levels. In particular, the contrast between phlorhizin and insulin, save for their effect on the blood sugar, supports the hypothesis that hyperglycemia plays a part in the pathogenesis of diabetes. This hypothesis must be regarded as tentative until such facts as the following are more fully understood. Hydropic degeneration has not been seen in the islands of the diabetic rat. Other species have not been studied and ought to be better understood before the results in the cat and dog are accepted as final. Mild diabetes in animals (and man) may persist for long periods without rapid progression in the severity of the disease. This is in contrast to the severe glycosuria produced during the period of pituitary treatment. The minor anatomical changes after glucose perfusion are not comparable to the frank lesions of experimental diabetes. In the authors' opinion the weight of evidence appears to favor the concept that the level of blood glucose is a factor of importance in the pathogenesis or recovery of diabetes.

SUMMARY

Further experiments with pituitary-diabetes in partially depancreatized cats have confirmed our previous observations on the production, course and manifestations of this condition.

Treatment of the diabetes by phlorhizin, if begun within the first 3 months, has been followed by morphological restoration of the islands and functional recovery of the animals. After 3 months of diabetes, when atrophy of the pancreatic islands had developed, there was no recovery after the administration of phlorhizin. This agrees with our previous experience with insulin and dietary treatment given late in the disease. Injury to the islands of Langerhans was prevented when phlorhizin was administered simultaneously with diabetogenic anterior pituitary extract. Phlorhizin abolished hyperglycemia without inhibiting gluconeogenesis, ketogenesis or thyrotropic activity. The action of phlorhizin and that of insulin have been compared. In the diabetic animal they appeared to differ in most respects. The

only clear similarity was in their action on the blood-sugar level and the concomitant benefit to early diabetes. In this connection the possible rôle of the level of blood glucose in the pathogenesis of diabetes has been reviewed.

REFERENCES

ALLEN, F. M.: Glycosuria and Diabetes. W. M. Leonard, Boston. 1913.
ALLEN, F. M.: *J. Metab. Res.* 3: 623. 1923.
BECK, L. V.: *Proc. Soc. Exper. Biol. & Med.* 49: 435. 1942.
COPP, E. F. F., AND A. J. BARCLAY: *J. Metab. Res.* 4: 445. 1923
DEUEL, H. J., H. E. C. WILSON AND A. T. MILHORAT: *J. Biol. Chem.* 74: 265. 1927
DOHAN, F. C., AND F. D. W. LUKENS: *Am. J. Physiol.* 122: 367. 1938.
HAIST, R. E., AND C. H. BEST: *Science* 91: 410. 1940.
HEDON, E.: *Compt. rend. Soc. Biol.* 4: 60–62. 1897.
HOUSSAY, B. A., AND V. G. FOGLIA: *Revist. Soc. Argent. Biol.* 13: 370. 1937.
LEPINE, R.: Le diabete sucre. Felix Alcan. Paris. 1909.
LUKENS, F. D. W., AND F. C. DOHAN: *Endocrinology* 30: 175. 1942.
MINKOWSKI, O.: Untersuchungen über den Diabetes mellitus nach Extirpation des Pancreas. Leipzig. 1893.
MIRSKY, I. A., J. D. HEIMAN AND R. H. BROH-KRAHN: *Am. J. Physiol.* 118: 290. 1937.
NASH, T. P.: *Physiol. Rev.* 7: 385. 1927.
SOSKIN, S., R. LEVINE AND W. LEHMANN: *Proc. Soc. Exper. Biol. & Med.* 39: 442. 1938.

ADRENOCORTICAL-LIKE TISSUE IN THE OVARIES OF THE ADRENALECTOMIZED GROUND SQUIRREL (CITELLUS TRIDECEMLINEATUS)[1,2]

RICHARD A. GROAT

From the Department of Anatomy, University of Wisconsin
MADISON, WISCONSIN

IN A PREVIOUS paper it was stated that the ground squirrel survives adrenalectomy if the food intake is maintained, which occurs if the animal is allowed to drink 1 per cent sodium chloride solution (Groat, 1941). A lessened food intake and the inability to survive a fast are good criteria of two different levels of adrenal insufficiency in this animal. At a variable time after adrenalectomy (minimum, 15 days for females), 1 per cent sodium chloride solution may be replaced by water and the animal will continue to eat; but it will not be able to survive a fast until some 3 or 4 months after operation. The nature of this gradual physiologic adjustment has proved to be the appearance and growth of adrenocortical-like tissue in the animal. The ovary is an invariable site for the manifestation of this phenomenon.

MATERIAL

The material for this report consists of serial sections of the ovaries of 46 ground squirrels. The ovaries were fixed in some variation of a mercuric chloride-potassium dichromate-formalin mixture. That most commonly used was an aqueous solution of 5 per cent mercuric chloride and 2.5 per cent potassium dichromate to which was added 1 per cent formalin just before use. The sections were stained with a tetrachrome stain (Groat, unpublished).

Seven of the ground squirrels were normal, one was normal but fasted, 34 were adrenalectomized, and 4 were hypophysectomized and adrenalectomized. Estrous and pregnant animals were not included in the study. Some received only water to drink after adrenalectomy, and these fasted voluntarily even though they had access to food; some received 1 per cent sodium chloride solution with and without food; some were fasted beginning a month after the operation; and some received a ration other than the one regularly used. There was no difference in the metaplasia in the ovaries among any of these subgroups.

Adrenalectomy was performed as a one-stage operation. Hypophysec-

Received for publication March 10, 1943.
[1] Part of this work was done at the University of Wisconsin, aided by a grant from the Wisconsin Alumni Research Foundation.
[2] Present address, Institute of Neurology, The Medical School, Northwestern University, Chicago, Illinois.

tomy was accomplished by an oral approach, and was followed by adrenalectomy a day later.

Adrenalectomized animals were autopsied from 3 days to one year after operation. Only a few of these succumbed to adrenal insufficiency, and then within a month after operation. Hypophysectomized and adrenalectomized animals were killed from 3 to 9 days after adrenalectomy.

RESULTS

Study of microscopic sections indicated that cells resembling adrenocortical cells in the ovaries of adrenalectomized ground squirrels originated almost entirely by metaplasia of certain cells in the ovaries and in the region of the mesovarium attached to them. The hypophysis is involved in the process.

A detailed histologic description of the ovary of the ground squirrel relevant to the inception and growth of adrenocortical-like tissue in it will be published elsewhere.

Metaplasia in the ovary began soon after adrenalectomy. In all cases observed, both ovaries were essentially the same. In an animal 3 days after operation single adrenocortical-like cells and small clumps were found scattered about the medulla of the ovary, and aggregations of these cells were found in the mesovarium at the hilus. In the medulla of the ovary the adrenocortical-like cells seemed to be derived from undifferentiated cells and from fibroblasts. In the mesovarium they appeared to come from embryonic cells associated with the epoöphoron, and later also from fibroblasts and undifferentiated cells at the periphery of aggregations already formed, and occasionally from fibroblasts and undifferentiated cells elsewhere.

New clumps were formed as time progressed. The clumps seemed to grow by differentiation at their periphery and by coalescence. Mitosis was limited. After 3 or 4 weeks the process of metaplasia had advanced well into the cortex of the ovary. Here, the precursors of the adrenocortical-like cells seemed to be fibroblasts and undifferentiated cells. In some cases there was evidence for formation from the cells of the tunica albuginea, and in one instance cells appeared to come from the germinal epithelium.

In an animal 118 days after adrenalectomy the medulla of the ovary was solidly filled with adrenocortical-like tissue, there were clumps in the cortex, and one large body swelled the mesovarium and extended into the ovary. Follicles and corpora lutea interfered with the process of metaplasia only in that these physically limited its spread.

No adrenocortical-like cells were present in the ovaries or in the mesovarium of any of the 4 hypophysectomized and adrenalectomized animals observed.

DISCUSSION

The evidence for the designation of certain tissue in the ovaries of adrenalectomized ground squirrels as adrenocortical-like is both morphologic and physiologic.

The cells in question cytologically resemble cells of the post-secretory zone of the adrenal cortex. Typically, they are large cells with dense, finely granular cytoplasm and a large, spherical, semichromatic nucleus which has one or two large, eccentrically located nucleoli. They appear to be exactly the same cell type as the one in accessory cortical bodies which occasionally occur in adrenalectomized ground squirrels and rats. The appearance of lipoid vacuoles in cells of the post-secretory zone of the adrenal cortex is inconstant. Hence, the fact that none appear in the extra-adrenal cortical cells is not unusual. Agate and Zwemer (1935) recognized that highly active extra-adrenal cortical tissue may not have the accepted appearance, and refer to a statement of Zwemer that this may be due to an exhaustion atrophy in which the cell products are used and the cells destroyed at a greater rate than they can regenerate and accumulate lipoid material. This may be an extreme statement, but it does seem that highly active extra-adrenal cortical cells are more apt to resemble post-secretory than secretory cells of the adrenal cortex.

In the serially sectioned ovaries of 8 normal ground squirrels, no trace of the adrenocortical-like cells has been found. In the serially sectioned ovaries of 34 adrenalectomized ground squirrels, the adrenocortical-like cells have been found in every case. Each of these 34 animals was searched carefully at autopsy for accessory adrenal tissue. A tiny cortical body was found near the kidney of one animal one year after adrenalectomy. Eleven of these adrenalectomized animals were kept alive sufficiently long to allow replacement of the 1 per cent sodium chloride solution by water, and were autopsied from 30 days to one year afterwards. Two of these 11 animals were subjected to a 14-day fast several months after adrenalectomy. They survived like normal animals.

On the basis of the above evidence, it was concluded that the cells functioned as adrenal cortex, at least to the extent of maintaining life and protecting against the deleterious effects of a fast. It might be expected that the growth and function of these cells, like the adrenocortical cells, would be controlled by the hypophysis. Study of the serial sections of the ovaries of the 4 hypophysectomized and adrenalectomized animals revealed no adrenocortical-like cells whatever.

Zwemer, *et al.*, (1938) described the glandular cells of the adrenal cortex as originating from the spindle-shaped fibroblast-like cells of the capsule of the adrenal. Later, Salmon and Zwemer (1941) demonstrated this graphically by injecting trypan blue into living rats. Ingle and Higgins (1938) showed that regeneration of adrenal autografts takes place only from the capsule, and concluded that this is dependent upon the adrenotropic activity of the anterior lobe of the hypophysis. The writer has seen indubitable evidence for this origin of adrenocortical cells in the rat and in the ground squirrel.

In the ground squirrel, hypophysectomy causes extreme atrophy of the adrenal cortex. Treatment with anterior pituitary substances brings about restoration to normal, presumably due to adrenotropic content (Zalesky, *et al.*, 1941).

Ingle, *et al.*, (1938) found that large doses of cortin produce atrophy of

the adrenal cortex in the rat, but not when an adrenotropic fraction of the anterior lobe of the hypophysis is given at the same time. Compensatory hypertrophy of the remaining adrenal after unilateral adrenalectomy has been an established fact for a long time. Cortin suppresses this compensatory hypertrophy (MacKay and MacKay, 1938). It is possible that the amount of adrenotropic factor secreted by the pars distalis is a function of the amount of cortin which is available to the organism.

In the experiments here reported it is assumed that the removal of the adrenals resulted in hypersecretion, or in accumulation in the blood of the adrenotropic factor of the hypophysis and that this elevated level of the adrenotropic factor in the blood effected the metaplasia in the ovary.

The ovary of the adrenalectomized rat does not produce adrenocortical-like cells. In this animal, food intake is still dependent upon sodium chloride intake 4 months after adrenalectomy (Groat, 1941). It is evident that extra-adrenal cortical cells do not occur, at least, in the strain of rats used by the writer.

It is probable that the formation of adrenocortical-like tissue in the ovaries does not occur physiologically in other common laboratory animals since the phenomenon has not been reported. The occurrence of this in the ground squirrel may be explained on the basis that the level of the adrenotropic factor in the blood following removal of adrenocortical tissue is higher. Linked with this possibility may be a greater sensitivity of the reacting cells. Experiments are now in progress to test the possibility of stimulating the production of adrenocortical-like tissue in other animals by administration of adrenotropic factor.

Formation of adrenocortical-like cells may occur elsewhere than in the ovaries. This is indicated by the results of removal of the ovaries before adrenalectomy and at various times afterwards. The metaplasia appears to occur more readily in the ovaries than elsewhere. On the basis of survival and resistance to fasting, metaplasia seems to occur also in the adrenalectomized male ground squirrel, but whether there is any constant locus as in the female has not yet been determined. Small cortical bodies have been found near the kidney long after adrenalectomy in one female and in three males. In these cases the cells seem to have arisen by metaplasia of fibroblasts.

We have found no evidence that such things as cortical rests occur in the ground squirrel.

SUMMARY

Following adrenalectomy, the ovaries of the female ground squirrel invariably become the site of formation of adrenocortical-like tissue. The process is principally one of metaplasia, and does not occur in the absence of the hypophysis.

Cytologically, the adrenocortical-like cells of the ovary resemble adrenocortical cells. Physiologic evidence for the identity of these cells is that they have not been found in the ovaries of normal ground squirrels, and without exception have been found in the ovaries of adrenalectomized animals. In

only one of 34 adrenalectomized females was a tiny body of adrenocortical-like tissue found elsewhere; yet these animals, when permitted, remained in good condition and were protected against the effects of fasting without receiving treatment. The fact that hypophysectomy prevents the metaplasia is corroborative evidence.

I wish to acknowledge the kindness of Dr. H. W. Mossman, which made this work possible; and the aid of Mr. Alex Newman in the hypophysectomy operations.

REFERENCES

AGATE, F. J., AND R. L. ZWEMER: *Am. J. Physiol.* 111: 1. 1935.
GROAT, R. A.: *Am. J. Physiol.* 135: 58. 1941.
INGLE, D. J., AND G. M. HIGGINS: *Endocrinology* 22: 458. 1938.
INGLE, D. J., G. M. HIGGINS AND E. C. KENDALL: *Anat. Rec.* 71: 363. 1938.
MACKAY, E. M., AND L. L. MACKAY: *Endocrinology* 23: 237. 1938.
SALMON, T. N., AND R. L. ZWEMER: *Anat. Rec.* 80: 421. 1941.
ZALESKY, M., L. J. WELLS, M. D. OVERHOLSER AND E. T. GOMEZ: *Endocrinology* 28: 521. 1941.
ZWEMER, R. L., R. M. WOTTON AND M. G. NORKUS: *Anat. Rec.* 72: 249. 1938.

OXYGEN CONSUMPTION OF SKIN AND HAIR GROWTH AFTER ADRENALECTOMY IN THE WHITE RAT

EARL O. BUTCHER

From the Biological Laboratory, Hamilton College
CLINTON, NEW YORK

IT HAS BEEN pointed out elsewhere (Butcher, 1937) that hair growth is accelerated by bilateral adrenalectomy either in well fed or underfed rats. Since oxidative processes are believed to be important in favoring growth in active hair follicles (Butcher, 1943), and adrenal extract depresses the oxygen consumption of brain slices (Tipton, 1939) and decreases the oxygen consumption of human beings under certain conditions (Hitchcock, *et al.*, 1938), this accelerated growth after adrenalectomy may be due to increased respiration of the skin.

To test the above hypothesis, determinations of the oxygen consumption of the skin were made using underfed rats at various intervals following adrenalectomy.

METHOD

Rats were weaned at the age of 22 days, and thereafter given 1 per cent NaCl solution as drinking water and the daily quota of 4 gm. of finely ground Purina dog chow. Such underfed rats maintained a body weight of 40 to 50 gm. Underfed rats were used since their skin consumed a similar amount of oxygen for many consecutive days. The oxygen consumption of the skin of well fed rats, on the other hand, changed frequently (Butcher, 1943), and an increase in oxygen consumption of the skin after the removal of the adrenals might not be the result of adrenalectomy. Hair follicles remained inactive in underfed rats, and they were induced to grow by bilateral adrenalectomy.

Hair was removed with sodium sulphide from both the dorsum and the venter of underfed rats, and bilateral adrenalectomy then performed from the ventral approach so that injury would not affect the oxygen consumption of the skin of the dorsum. Rats were killed at various intervals following adrenalectomy, and the oxygen consumption of the skin was measured volumetrically in air.

Fenn microrespirometers were used as described previously (Butcher, 1943) in making oxygen determinations[1]. The tissues were shaken in Ringer's-phosphate solution (pH 7.2) at a temperature of 37.5°C. for 2 hours. After making the tests, the tissues were dried to a constant weight at 110°C. Usually four respirometers, each containing 4 strips of skin (1 cm. long and

Received for publication March 24, 1943.

[1] I am indebted to Dr. J. A. Dye of the Physiology Department of Cornell University for the use of microrespirometers.

.5 mm. wide) from the back of the rat, were shaken at the same time. Results are expressed in cubic millimeters of oxygen consumption per milligram of dry tissue per hour.

RESULTS

The oxygen consumption of the skin from the backs of 13 underfed and unoperated animals (40 determinations) averaged .92 cu.mm. per mg. of dry tissue during the first hour. During the second hour that the same micro-respirometers were run the average for the same 13 rats (34 determinations) was .85 cu.mm. The average consumption for the first and second hours per mg. of tissue, as shown in figure 1, was .88 cu.mm. The skin of these underfed

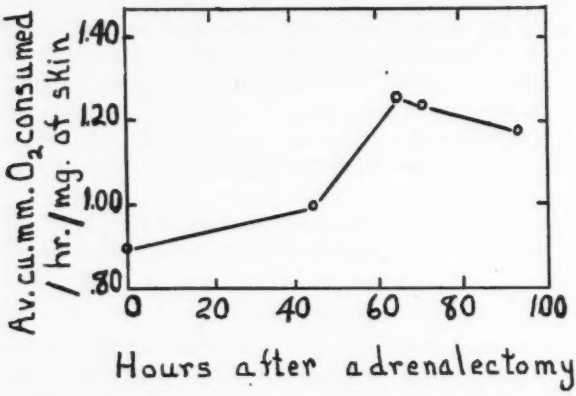


Fig. 1. Effect of adrenalectomy on the oxygen consumption of the skin.

rats contained only inactive follicles and the oxygen consumption was comparable to that used by skin of well fed rats containing resting hair follicles (Butcher, 1943).

Twenty-one determinations were made on 6 different rats which had been adrenalectomized 46 hours previously. The average oxygen consumption of the skin per mg. of dry tissue for the first hour was 1.10 cu.mm. or approximately a 20 per cent increase over that of the skin of the unoperated rat. During the second hour the average for 18 determinations on the same 6 rats was .89 cu.mm. per mg. The average of the determinations for the first and second hours was 1.00 cu.mm. per mg., a 14 per cent increase over the oxygen consumption of the skin of underfed and unoperated animals.

The hourly oxygen consumption of the skin of 6 other rats which had been adrenalectomized 66 hours previously averaged 1.25 cu.mm. per mg. for 29 determinations (fig. 1). The same high rate of O_2 consumption existed 72 (31 determinations in 6 rats) and 96 hours (24 determinations in 4 rats) after adrenalectomy. Hair buds seldom become active before 72 hours after adrenalectomy. Oxidative processes thus increase prior to activity in the follicle.

It is interesting to note that the consumption of 1.25 cu.mm. of oxygen per mg. recorded 66 hours after adrenalectomy and prior to active hair growth compares favorably with the consumption of 1.21 cu.mm. of oxygen in the normal 31-day-old animal just prior to the regular cyclic hair growth (Butcher, 1943).

DISCUSSION

Investigators differ about the effects of adrenalectomy on the basal metabolism of rats. Tipton (1940) states that the basal metabolism of rats is depressed after adrenalectomy. Carr and Beck (1937), on the other hand, find that there is a marked rise in metabolism 1 to 4 days after adrenalectomy and preceding death in rats. Over the entire survival period of the adrenalectomized rats they report a 25 per cent decrease in the total metabolism. The present experiments show that an increase in the oxidative processes of the skin occurs very soon after adrenalectomy which confirms the 'marked rise in metabolism' within 1 to 4 days after adrenalectomy observed by Carr and Beck.

Regarding the effect of cortical hormones on the respiration of tissues, Tipton (1939) finds that corticosterone, extracts of the adrenal whole gland, or extracts of adrenal cortex have a depressing action on the oxygen consumption of brain and liver slices of normal and adrenalectomized rats. These results indicate that the cortex of the adrenal has an inhibitory effect on tissue oxidation.

Crimson and Field (1940) determined the respiratory rates of tissues 4 to 8 days after adrenalectomy. There was no difference between the respiratory rate of brain from normal and adrenalectomized groups of rats. No difference was found in the oxygen consumption of skeletal muscle until the 7th or 8th day after the operation. Oxygen consumption of kidney slices was reduced 50 per cent the sixth day after adrenalectomy. Tipton (1940) found that the rate of oxygen consumption of liver and kidney slices from rats showing marked symptoms of adrenal insufficiency 9 days after adrenalectomy was significantly depressed. So far as the author is aware no observations have been made on the oxygen consumption of tissues prior to the fourth day following adrenalectomy or during the interval of 46 and 66 hours after adrenalectomy when the present experiments were conducted. During this period there was a marked rise in oxidative processes in the skin.

SUMMARY

Experiments were conducted to determine if hair growth following adrenalectomy was related to an increase in the O_2 consumption of the skin.

Determinations were made with Fenn respirometers and in Ringer's-phosphate solution at 37.5° C. Skin of underfed and unoperated animals containing inactive hair follicles used about .9 cu.mm. of oxygen per mg. of dry tissue per hour. The oxygen consumption of skin of rats which had been adrenalectomized 46 hours previously increased 14 per cent over this value. Sixty-six hours after adrenalectomy and just prior to the beginning of growth

in the resting hair follicle, the oxygen consumption of the skin of another group of rats reached 1.25 cu.mm. (39 per cent increase) per mg.

Increased oxygen consumption of the skin results from adrenalectomy in the white rat, and these increased oxidative processes appear to induce hair growth in inactive follicles.

REFERENCES

BUTCHER, E. O.: *Am. J. Physiol.* 120: 427. 1937.
BUTCHER, E. O.: *Am. J. Physiol.* 138: 408. 1943.
CARR, C. J., AND F. F. BECK: *Am. J. Physiol.* 119: 589. 1937.
CRIMSON, J. M., AND J. FIELD, 2d: *Am. J. Physiol.* 130: 231. 1940.
HITCHCOCK, F. A., R. C. GRUBBS AND F. A. HARTMAN: *Am. J. Physiol.* 121: 542. 1938.
TIPTON, S. R.: *Am. J. Physiol.*: 127: 710. 1939.
TIPTON, S. R.: *Proc. Soc. Exper. Biol. & Med.* 45: 596. 1940.

EFFECT OF DIFFERENCES IN LIGHT AND TEMPERATURE UPON THE SIZE OF COMBS ON WHITE LEGHORNS[1]

W. F. LAMOREUX
Cornell University
ITHACA, NEW YORK

THE COMBS on Leghorn males, poulards and capons have been found to attain larger size when kept indoors in the absence of direct sunlight than when the fowls have access to outdoor pens. The combs on ovariotomized fowl were observed by Domm (1930) to increase greatly in size in the winter, a change believed to result from a deficiency of sunlight. Later, when extracts containing male sex hormone were assayed in two different laboratories using comb growth in capons, the results were not the same. In subsequent studies Womack, Koch, Domm and Juhn (1931) showed that when Brown Leghorn capons had access to direct sunlight, the combs responded to injections of male sex hormone with less growth than did the combs of birds confined indoors. White Leghorn cockerels, studied by Buckner, Insko and Martin (1932, 1933), developed much larger combs and wattles when reared indoors than when they had access to outdoor yards. Because of these facts and because of the undesirable excessive growth of combs on cockerels reared commercially in confinement, further knowledge of the relationship between environment and growth of the comb is needed.

The present experiment was undertaken to study the relative effects of *a*) ultraviolet light, *b*) long and short daily periods of light, and *c*) differences in environmental temperature, upon the growth of combs on White Leghorn cockerels.

Ultraviolet Light

To test the effectiveness of ultraviolet light in retarding comb growth, two experiments were conducted in which chicks were irradiated with light from an S 4, A-H 4, or an ordinary 150-watt lamp, 14 hours daily. The S 4 (ultraviolet) lamp was suspended about 5 feet above the wire cage in which the chicks were confined. The A-H 4 lamp, similar to the S 4 except that the glass bulb filters out the ultraviolet light, and the 150-watt lamp were so placed that intensity of light upon the chicks, measured with a Weston photometer (Model 715), was equal to that from the S 4 lamp. The three lamps and three groups of chicks were placed in adjoining compartments separated from each other by 2 layers of heavy red cloth. The cages were approximately 30″ square and 10″ deep, small enough that the chicks could not avoid uniform irradiation. The compartments were open on the front to provide ven-

Received for publication April 2, 1943.
[1] Aided by a grant from the Cornell University Trustee-Faculty Committee on Research.

tilation. The A-H 4 lamp was placed in the middle compartment to avoid risk of ultraviolet light being reflected to the chicks irradiated with the 150-watt lamp. The room temperature was thermostatically controlled and ranged between 83° and 90°F. at the top of the cages. However, the average temperature was about 2°F. lower in the cage exposed to the S-4 lamp than in the other two.

After a preliminary series of experiments which indicated the feasibility of the project, a second experiment was undertaken with larger numbers of chicks. Of these 19, 18 and 17 survived exposure to the S 4, A-H 4 and 150-watt lamps, respectively. These cockerels were placed on experiment when 2 weeks of age. At 7 weeks of age, cage size being inadequate, the chicks were moved to standard laying cages in three dark rooms. These were so insulated that light could not pass from one pen to another. In order to irradiate all birds uniformly the S 4 lamp was placed about 10 feet from the cages and the light was projected horizontally. The A-H 4 and 150-watt lamps were again arranged to provide intensities of light in the cages equal to that from the S4 lamp. The mean temperatures in the rooms containing the S4, A-H 4 and 150-watt lamps were 69, 72 and 70°F., respectively. The temperature ranged between 61 and 77°F.

After 11 weeks of experimental treatment, when the cockerels were 13 weeks of age, the average comb size of the three groups did not differ significantly (fig. 1). The birds exposed to ultraviolet light had the smallest combs and those exposed to the 150-watt lamp had the largest, just as in the previous experiment. However, the differences were small and showed that ultraviolet light is only slightly effective, if at all, in retarding growth of combs on cockerels less than 13 weeks of age.

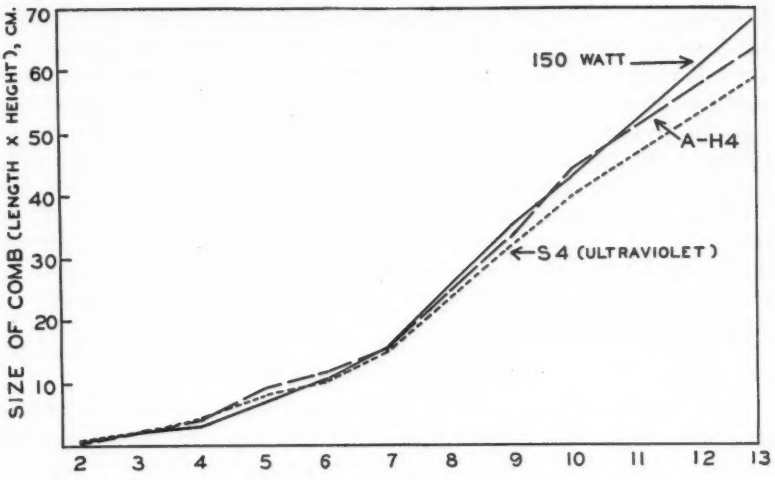
Part of the differences which occurred may have resulted from differences in the production of hormones by testes of different size. The average weights

TABLE 1. AVERAGE WEIGHT OF TESTES FROM GROUPS OF CHICKS EXPOSED TO LIGHT

TREATMENT	EXP. I	EXP. II (FIG. 1)
	gm.	gm.
S 4 (ultraviolet)	1.3	2.8
A-H 4	2.1	2.8
150-watt	2.5	4.2

of testes from groups exposed to these lamps are given in table 1. It has been shown by Greenwood and Crew (1927), Blyth (1928), Breneman (1938) and Jones (1943) that growth of comb in cockerels is associated with the size of the testes, development of seminiferous tubules or spermatogenesis. Actually the size of testes in the first and third groups of cockerels differed to a greater degree than did their combs.

These results are similar to those obtained with starlings by Bissonnette and Wadlund (1933) who found that a 'G. E. sunlamp' provided less stimulation of testicular development than did a 200-watt lamp of equal luminous intensity. The relatively small testes of birds exposed to ultraviolet light may be the result of inadequate exposure to visible red light. It seems unlikely that ultraviolet light has a direct inhibitory effect upon the testes because Buckner,



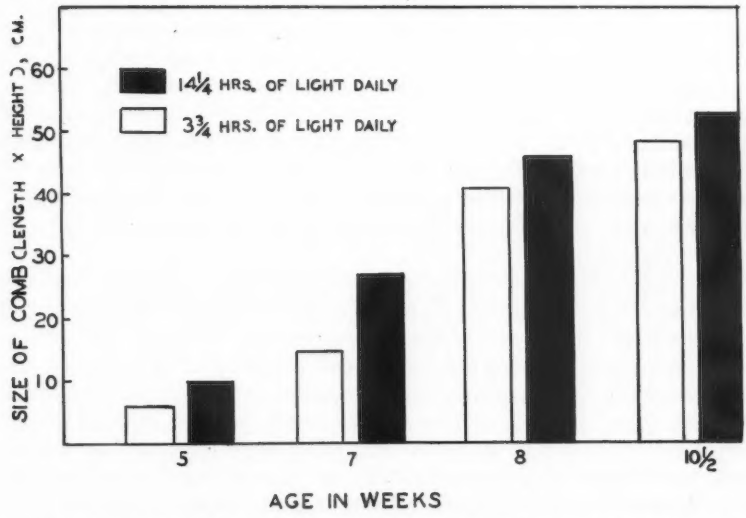


Fig. 1, (*upper*). Average size of combs on White Leghorn cockerels exposed to light from an S 4 (ultraviolet), A–H 4, or ordinary 150-watt lamp. The differences were not significant. Seventeen to 19 chicks in each group.

Fig. 2, (*lower*). Average size of combs on White Leghorn cockerels exposed to 14.25 hours of light daily, or to 1.25 hours of light every 8 hours. A dim light, 7.5-watt, was burning 15 minutes after the bright lights (75-watt) were turned out. The difference in comb size was insignificant at 10.5 weeks of age.

Insko and Martin (1932) and Nikolaiczuk and Maw (1942) found larger testes in Leghorn cockerels exposed to direct sunlight than in those which were not.

Direct evidence that the sensitivity of the comb to measured amounts of

hormone can be modified by differences in light, and perhaps temperature, has been provided by studies of comb growth in capons (Womack, *et al.*, 1931; Koch and Gallagher, 1934). That such differences in sensitivity result primarily from the direct effect of ultraviolet light upon the comb now seems unlikely. In the present study ultraviolet light from the S 4 lamp did not inhibit comb growth significantly (fig. 1). Combs irradiated with ultraviolet light were slightly smaller during most of the experiment, but this difference is best explained by the difference in testis size. In view of the intense radiation (lamp 5 feet from chicks, 2–7 weeks; 10 feet, 8–13 weeks) during 14 hours daily, more striking differences would be expected if ultraviolet light were the principal cause of the relatively small combs on males reared out-of-doors.

Different Daily Periods of Light

Fifty male chicks were placed in each of two dark pens (10×14 ft.) where they were exposed to 14.25 or 3.75 hours of light daily. To enable the chicks to learn to eat and drink, light was provided throughout the first night after they were placed under the brooder. Thereafter, one group was exposed to the light from a 75-watt lamp 14 hours daily, whereas the other received one hour of such light every 8 hours. After the bright light was turned off, dim light from a 7.5-watt lamp was provided for 15 minutes to enable the birds to find roosts. The lights were controlled by automatic electric time switches. Adequate space was provided at the feeders so that all of the birds could eat at one time. During 6 weeks of the experiment the room temperature, recorded daily, ranged between 63 and 84°F. The average temperatures in the two rooms were 70 (3.75 hours of light) and 71°F. (14.25 hours of light). The chicks were brooded under electric brooders from which the pilot lights had been removed.

The average size of combs on these cockerels at 5, 7, 8 and 10.5 weeks of age is shown in figure 2. At each age those males exposed to most light had larger combs, but at the end of the experiment this difference was not significant ($P = >0.05$). During the first 7 weeks chicks exposed to 14.25 hours of light were slightly heavier; thereafter the relative weights were reversed. After fasting, and just before they were killed the cockerels exposed to 3.75 hours of light daily had an average weight of 1,048 gm., compared to 976 gm. for the others. This difference makes it clear that the relatively small combs of the birds exposed to least light did not result from reduced consumption of feed because of darkness.

The average weights of testes from the cockerels exposed to 14.25 and to 3.75 hours of light daily were 2.15 and 1.60 gm., respectively. This difference is not significant ($P = >0.05$), but in view of the highly significant coefficient of correlation ($r = 0.48$) between testis size and size of comb, within groups, it could readily account for the difference in comb size.

Fiske (1939) found that when rats were continuously exposed to light the testes were 30 per cent heavier and the pituitaries almost twice as large as those of males kept in darkness. In the present study the cockerels exposed to

3.75 or 14.25 hours of light daily had testes that weighed 1.60 and 2.15 gm.; combs, 16.0 and 19.4 gm.; and anterior pituitary glands, 7.2 and 7.4 mg., respectively. The size of testes in these cockerels differed slightly more than in the rats; the pituitaries differed much less.

Domm (Womack, *et al.*, 1931) proposed that exercise may have some effect upon comb growth. Later Koch and Gallagher (1934) reported that the response of capons to testicular hormones during continuous illumination following 11 days or longer of constant darkness is markedly reduced. A similar

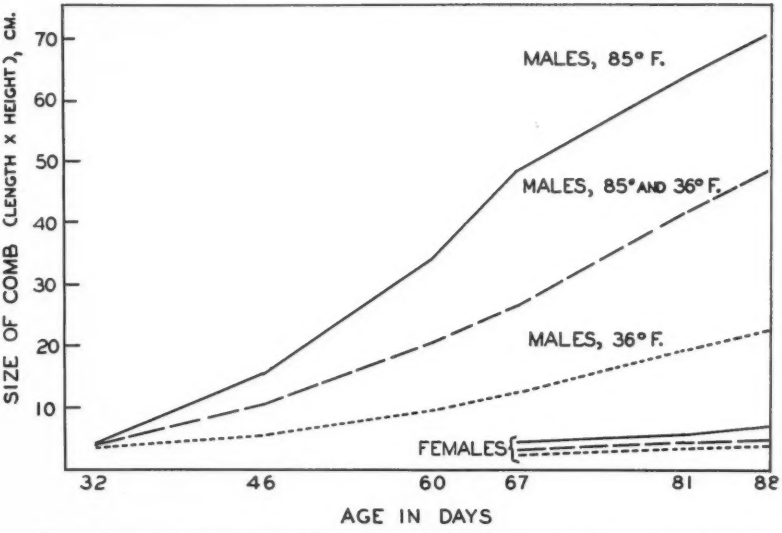


Fig. 3. Average size of combs on White Leghorn chicks exposed to environmental temperatures of 85 and 36°F., or transferred from one temperature to the other every 12 hours. Differences among the males were highly significant. Five chicks in each group.

reaction may be involved in recent evidence that Leghorn males make significantly greater gains in body weight when exposed to light 9 hours or less daily, than when exposed 12 hours or more (Lamoreux, 1943 a). Birds previously kept in darkness are likely to be abnormally active for a time after exposure to continuous light so that actual loss of body weight may occur. Such circumstances, rather than the direct effect of light upon the comb, may explain the limited response of the comb obtained by Koch and Gallagher.

Environmental Temperatures

The effect of different temperatures upon comb growth was studied by placing one group of 10 chicks in a room maintained at 85°F., another group in a refrigerated room at 36 °F., and by changing a third group from one room to the other every 12 hours. The chicks were 32 days of age at the beginning of the experiment, and each group consisted of 5 males and 5 females, selected

on the basis of the body weight. Because chicks of this age will normally huddle together to keep warm when the temperature is low, each chick was placed in a wire cage 11 inches high and 10 inches in diameter. When the birds were 65 days of age they were transferred to individual laying cages of a standard type.

These birds were exposed to light from 75-watt lamps during 14 hours each day. The intensity of the light at the position of the birds in the warm room ranged between 1.5 and 6.5 foot candles; in the cold room it ranged between 1.5 and 3.5 foot candles. Such light is believed to be of more than adequate intensity to stimulate maximum development of the testes and spermatogenesis in the fowl. Roberts and Carver (1941) have shown that one-foot

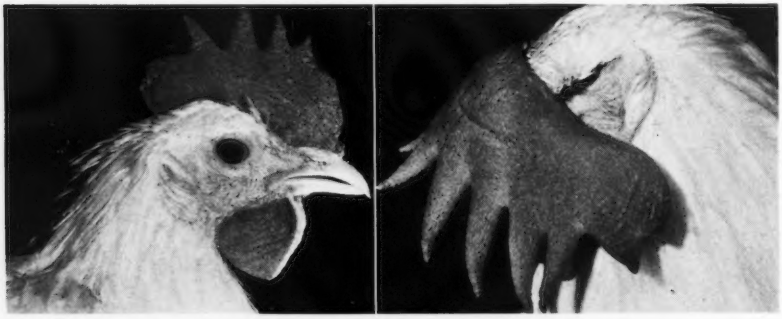


Fig. 4. Combs on cockerels 88 days of age, after 56 days of exposure to environmental temperatures of 36°F. (small comb) or 85°F. (large comb). These combs were nearly the same size as the average for their respective groups (length×height: small comb, 22.4 sq. cm.; large comb, 68.2 sq. cm.), cf. figure 3. These birds weighed 1,380 (*left*) and 1,200 gm. (*right*).

candle of light is as effective as 30-foot candles for stimulating egg production in Leghorn hens, and Lamoreux (1943 b) found 0.2 foot-candle of light adequate to maintain yields of semen in mature Leghorn males. Electric fans ran continuously over the cooling coils of the cold room, but only periodically in the thermostatically controlled heater in the warm room. Relative humidity could not be controlled readily, but on several occasions when determined with a sling psychrometer it was found to be higher in the cool room.

The effect of these treatments upon comb growth in chicks from 32 to 88 days of age is shown in figure 3. Throughout the experiment there was a marked and increasing difference in average comb size in the three groups of cockerels. In figure 4 combs which are of the average size for the 5 males in the cold and warm rooms, respectively, are shown to illustrate the actual differences obtained. In each of these groups there were two males with larger and two with smaller combs than those illustrated. It is apparent from figure 3 that the combs on males subjected alternately to the high and low temperatures were intermediate in size between those shown in figure 4.

The effect of different temperatures upon the size of combs on females is much less conspicuous at the ages studied. Unfortunately, the refrigeration

equipment failed and a study of later ages was not possible. However, the small differences observed were in the same order as those found among males (fig. 3).

The great differences in size of combs among the three groups of males are apparently independent of body weight. The average weights of cockerels kept at 85, 36, or 85 and 36°F., respectively, were 1,277, 1,226 and 1,363 gm. at the end of the experiment.

Differences in environmental temperature may influence the size of the comb in at least three ways. First, although the cockerels exposed to different environmental temperatures did not differ significantly in body size, the testes of birds in 85, 85 and 36, or 36°F. temperatures weighed 5.2, 4.9 and 2.7 gm., respectively. Such differences would normally be expected to influence the size of combs on birds of this age (Jones, 1943). However, among those exposed to 85°F. the male with smallest testes (0.4 gm.) had a larger comb (48.7 sq. cm.) than the male with largest testes (5.0 gm.) and largest comb (30.2 sq. cm.) among those exposed to 36°F. Obviously the difference in temperature had some effect other than upon size of testes.

A second type of effect might result from differences in the production of hormone, irrespective of testis size. Greenwood and Crew (1927), Breneman (1938) and Jones (1943) found, respectively, that comb growth was associated with intense cellular activity, hypertrophy of the seminiferous tubules or spermatogenesis. Riley (1937, 1940) found spermatogenesis in the sparrow and the fowl to be most active when the body temperature was lowest. Macartney (1942) was unable to substantiate Riley's results in the fowl, but in view of the possible effect of internal body temperature upon the production of hormones by the testis it should be noted that the average rectal temperatures of the males exposed to 85°F. were lower in 10 of 12 morning or evening recordings near the end of the experiment, than were those of birds in the cold room. The differences did not exceed one degree, but such differences as occurred should tend to favor cellular activity in the testes of birds in the warm room, the birds with the largest combs.

A third means by which environmental temperature may affect the size of comb is its effect upon the temperature of the comb itself. Cockerels in the cold consumed more feed and maintained a slightly higher internal temperature to offset the increased loss of heat from the body. However, the comb and wattles of birds in the cold room were much lower in temperature than those of cockerels in the warm room. The quantitative effects of such low temperatures upon the chemical reactions concerned with response to androgenic hormone have not been measured directly, but it seems likely that much of the difference shown in figure 3 results from the relatively low cell temperature in the comb and the restricted peripheral circulation in males exposed to low temperatures.

Birds kept in confinement during the summer for experimental or commercial purposes are normally exposed to higher temperatures than are birds which have access to shade and breeze out-of-doors. The relatively high humidity commonly encountered when birds are kept in confinement would

accentuate the effects of high temperature by hindering loss of body heat, thereby increasing the peripheral circulation of blood and the temperature in the comb. During winter the differences between environmental temperatures inside and out-of-doors are still greater, and sometimes approach the range of 49°F. employed in this study. In view of the great differences in comb growth observed during an experimental period of only 8 weeks, it seems likely that much of the increased comb growth on cockerels reared in confinement results from the relatively high temperature, humidity, or both, to which they are exposed during long periods of time.

These results show that, of the methods studied, the most effective means of inhibiting growth of combs on cockerels confined indoors is to maintain the environmental temperature at a low level. Conversely, they also suggest that the growth response of the capon comb to androgenic hormone will be greatest when the environmental temperature is high, and maintenance of uniformly high temperature may be worth while for increasing the sensitivity and uniformity of results in the assay of activity of androgenic hormones.

SUMMARY

Results obtained in this study show *a*), that irradiation with ultraviolet light has little direct effect upon comb growth, *b*) that exposure to artificial light during short periods of time daily does not induce greater comb growth than long daily exposure to similar light, and *c*) that, of the methods studied, the most effective means of inhibiting growth of combs on cockerels confined indoors is to maintain the environmental temperature at a low level.

REFERENCES

Bissonnette, T. H., and A. P. R. Wadlund: *Bird-banding* 4: 8. 1933.
Blyth, J. S. S.: *Proc. Roy. Phys. Soc.* 21: 175. 1928.
Breneman, W. R.: *Indiana Univ. Publ., Sci. Ser.* 7: 1. 1938.
Buckner, G. D., W. M. Insko, Jr., and J. H. Martin: *Amer. J. Physiol.* 102: 271. 1932.
Buckner, G. D., W. M. Insko, Jr., and J. H. Martin: *Poultry Sci.* 12, 392. 1933.
Domm, L. V.: *Anat. Rec.* 47: 314. 1930.
Fiske, W. M.: *Proc. Soc. Exper. Biol. & Med.* 40: 189, 1939.
Greenwood, A. W., and F. A. E. Crew: *Proc. Roy. Soc. Edin.* 47: 190. 1927.
Jones, D. G.: Ph.D. thesis submitted to Cornell University, pp. 1–70. 1943.
Koch, F. C., and T. F. Gallagher: *J. Biol. Chem.* 105: xlix. 1934.
Lamoreux, W. F.: *Ecology* 24: 79. 1943 a.
Lamoreux, W. F.: *J. Exp. Zool.* (in press). 1943 b.
Macartney, E. L.: *Poultry Sci.* 21: 130. 1942.
Nikolaiczuk, N., and W. A. Maw: *Poultry Sci.* 21: 483. 1942.
Riley, G. M.: *Anat. Rec.* 67: 327. 1937.
Riley, G. M.: *Poultry Sci.* 19: 360. 1940.
Roberts, J., and J. S. Carver: *Agric. Engn.* 22: 357. 1941.
Womack, E. B., F. C. Koch, L. V. Domm and Mary Juhn: *J. Pharmacol. and Exper. Therap.* 41: 173. 1931.

A COMPARISON OF THE INFLUENCE OF SOME CRYSTALLINE HORMONES OF THE ADRENAL CORTEX ON THE DEPOSITION OF GLYCOGEN IN THE LIVER

ROGER M. REINECKE AND EDWARD C. KENDALL

From the Division of Experimental Medicine and Division of Biochemistry, Mayo Foundation

ROCHESTER, MINNESOTA

THE DEVELOPMENT of a method for the bioassay of the hormones of the adrenal cortex which influence the deposition of glycogen in the liver of the fasted rat (Reinecke and Kendall, 1942) has afforded an opportunity to compare the action of various crystalline steroids isolated from the adrenal cortex with each other, with desoxycorticosterone and with an extract of the gland. In the first experiment (fig. 1), the effect of a given dose of extract was compared with that of compounds A, B, E, G, H and 11-desoxycorticosterone acetate. The compounds were given in equal dosage by weight. Since the desoxycorticosterone acetate was dissolved in sesame oil, its action was compared with that of extract and compound E dissolved in this same oil. In the second experiment (fig. 2), the dosage of the desoxycorticosterone acetate was increased tenfold to bring out any trace of activity that it might possess. In the third experiment (table 1), larger groups of animals were used to assay compounds A, B and E in an attempt to determine whether there was any considerable difference among the potencies of these compounds.

The results indicate that glycogen was deposited in the liver in the presence of compounds A, B and E but that compounds G and H and 11-desoxycorticosterone acetate were without significant effect. Seven injections of 0.1 mg. per hour of compounds A, B or E had a potency of the same order of magnitude as 7 injections of the extract from 15 gm. of adrenal gland. The use of either sesame oil or physiologic salt solution as a solvent led to approximately the same result under the conditions used.

The results of the second experiment serve to emphasize the ineffectiveness of desoxycorticosterone acetate since even a tenfold increase in dosage did not lead to the deposition of a significant amount of glycogen in the liver. The third experiment failed to show a statistically significant difference in the amount of glycogen deposited under the influence of equal doses of compounds A, B and E, and indicates that there is probably less than a onefold difference among their respective potencies since the method was previously found to detect readily such a difference in dosage of the extract.

Received for publication April 12, 1943.

COMMENT

The demonstration that desoxycorticosterone acetate does not promote the deposition of glycogen in the liver is in agreement with the results of other investigations (Grattan and Jensen, 1940) and supports the thesis that only those hormones of the adrenal cortex which have an atom of oxygen at C_{11} influence carbohydrate metabolism (Kendall, 1941). The failure of com-

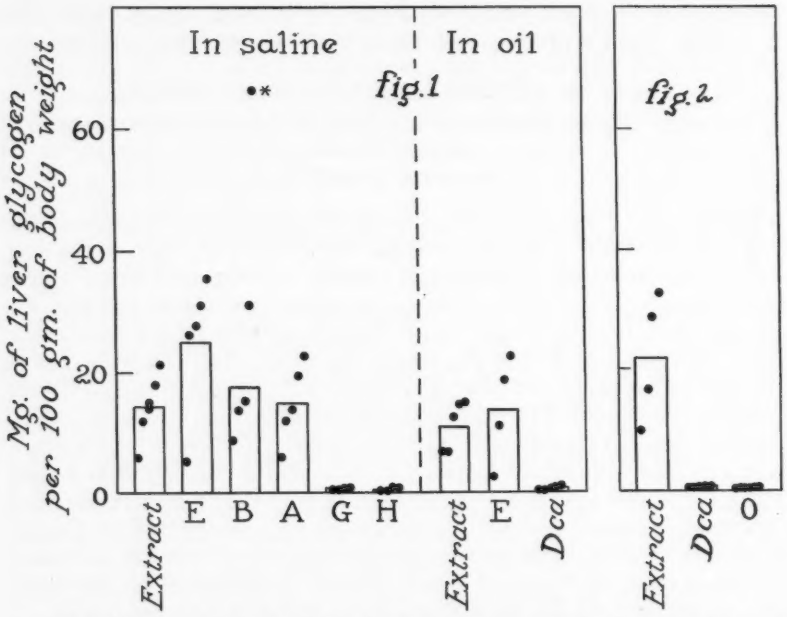


Fig. 1. Glycogen deposition in the liver. Bars indicate averages; points, individual values. Male rats, 142–186 gm. in weight, 4 days after adrenalectomy and fasted for one day, were given 7 subcutaneous injections of the preparations designated, at intervals of one hour. Between 7 and 8 hours after the first injection, they were anesthetized with 9 mg./100 gm. of body weight of sodium amytal intraperitoneally The livers were removed and analyzed for glycogen (Good, 1933). All animals were given 1 per cent salt solution to drink *ad libitum* throughout the experiment. The value for the point with the asterisk was not included in the average because of abnormal deviation. The preparations E, B, A, G and H were given in 0.1 mg. amounts in 1 cc. of physiologic salt solution per hour. The extract, from 15 gm. of beef adrenal gland, was given in 1 cc. of physiologic salt solution per hour. The extract in oil was from 15 gm. of beef adrenal gland in 0.2 cc. of sesame oil per hour. Preparations of E and DCA were each given in 0.1 mg. doses in 0.2 cc. of sesame oil per hour.

Fig. 2. The experiment was performed essentially as described in the legend of figure 1. The extract was from 15 gm. of beef adrenal gland in 0.2 cc. of salt solution per hour. The DCA was given in 0.1 mg. doses in 0.2 cc. of sesame oil per hour. 0 indicates that no injections were given.

pounds G and H to cause any appreciable deposition of glycogen in the liver, although they do have an atom of oxygen on C_{11}, may be ascribed to the absence of a ketone group at C_3 and a double bond Δ-4,5. Other studies on the physiologic effects of the hormones of the adrenal cortex have shown that those steroids that do not have the unsaturated ketone in ring A are inactive

(Long, *et al.*, 1940; Ingle, 1940; Wells and Kendall, 1940; Kendall, 1937). Compounds A, B or E have both an atom of oxygen at C_{11} and the unsaturated ketone in ring A. The similarity of their potencies to cause the deposition of glycogen may be correlated with the results of other studies which failed to show marked differences in their abilities to cause other effects (Long, *et al.*, 1940; Ingle, 1940).

Finally, it may be noted in passing that the potency of the extract used for comparison cannot all be accounted for by the amounts of compounds A, B and E that can be isolated from it. The combined weights of compounds A, B and E that can be isolated in crystalline form from an amount of extract

TABLE 1. INFLUENCE OF COMPOUNDS A, B AND E ON THE DEPOSITION OF GLYCOGEN IN THE LIVER

COMPOUND GIVEN	NUMBER OF ANIMALS	MG. OF LIVER GLYCOGEN PER 100 GM. OF BODY WT.	
		MEAN	STANDARD ERROR
A	10	10.3	±1.7
B	10	13.8	±2.4
E	11	15.0	±3.2

The experiment was performed essentially as described in the legend of figure 1. The animals were each given 0.1 mg. per hour of the compound indicated dissolved in 1 cc. of saline.

that gives approximately the same effect as 0.1 mg. of A, B or E, is about 0.03 mg. Since separation of the hormones in crystalline form under conditions which do not involve any demonstrable chemical change results in marked loss of physiologic activity, the explanation for the high potency of the extract cannot be stated in terms other than 'enhancement' of the activity of the crystalline hormones. This aspect of the potency of extract and crystalline hormones has recently been discussed (Kendall, 1942).

The compounds designated by letter which were used in this investigation are listed as derivatives of corticosterone.

A 11-Dehydrocorticosterone
B Corticosterone
E 17-Hydroxy-11-dehydrocorticosterone
G 17-Hydroxy-11-dehydro-3,4,5-tetrahydro(3β hydroxy)corticosterone
H 11-Dehydro-3,4,5-tetrahydro(3β hydroxy)corticosterone

SUMMARY

The influence of five crystalline steroids from the adrenal cortex, desoxycorticosterone acetate and an extract of the adrenal cortex on the deposition of glycogen in the liver of the fasting rat has been determined. Desoxycorticosterone acetate which lacks an atom of oxygen at C_{11}, and compounds G and H, which do not have an unsaturated ketone group in ring A, were without effect. Less than a onefold difference was found between compounds A, B and E.

REFERENCES

GOOD, C. A., H. KRAMER AND M. SOMOGYI: *J. Biol. Chem.* 100: 485. 1933.
GRATTAN, J. F., AND H. JENSEN: *J. Biol. Chem.* 135: 511. 1940.
INGLE, D. J.: *Endocrinology* 26: 472. 1940.
KENDALL, E. C.: Cold Spring Harbor Symposia on Quantitative Biology 5: 299. 1937.
KENDALL, E. C.: *Arch. Path.* 32: 474. 1941.
KENDALL, E. C.: *Endocrinology* 30: 853. 1942.
LONG, C. N. H., B. KATZIN AND EDITH G. FRY: *Endocrinology* 26: 309. 1940.
REINECKE, R. M., AND E. C. KENDALL: *Endocrinology* 31: 573. 1942.
WELLS, B. B., AND E. C. KENDALL: *Proc. Staff Meetings, Mayo Clin.* 15: 565. 1940.

DETERMINATION OF THE RATE OF THYROID HORMONE SECRETION AT VARIOUS ENVIRONMENTAL TEMPERATURES[1]

E. W. DEMPSEY AND E. B. ASTWOOD

From the Departments of Anatomy, Pharmacology and Medicine, Harvard Medical School

BOSTON, MASSACHUSETTS

THERE ARE many observations which suggest that the thyroid secretes its active principle more rapidly in cold than in warm environments. For example, physiological investigations have demonstrated increased metabolic rates after exposure to cold (Ring, 1939) and decreased rates after exposure to heat (MacGregor and Loh, 1941). Similarly, histological evidence of stimulation may be seen in thyroids removed from rats maintained in cold environments, while evidence of diminished secretion is apparent in glands of rats kept at high temperatures (Cramer, 1928). Moreover, it has been pointed out that hyperthyroid crisis is a more common clinical occurrence in geographical regions where the temperature shows wide fluctuations than in areas with a more gentle climate (Petersen, 1937). As these observations are based on diverse criteria and are in agreement that the thyroid gland is stimulated by cold, this conclusion may be accepted with considerable confidence.

It is a noteworthy fact, however, that this conclusion is based upon indirect evidence, from which only qualitative estimates of increased or decreased activity could be made. It is of little significance to inquire how much stimulation or depression is produced by a given situation unless it is possible to compare the degree of response to a normal value. Consequently, it appears that a method permitting the accurate determination of thyroid hormone production would be useful and provide interesting quantitative information.

The possibility of obtaining such quantitative information has been suggested by recently published observations on the mode of action of 'antithyroid' compounds (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell, and Tyslowitz, 1943; Astwood, 1943). These investigations have shown that certain compounds prevent the formation of the active thyroid principle. Because of this decrease or abolition of thyroid function, morphological evidence of stimulation of the thyroid gland results from the consequent over-activity of the pituitary thyrotropic factor. These experiments, therefore, lend support to the general endocrinological concept that normal conditions result from a balance of opposing forces. In this instance, the bal-

Received for publication April 22, 1943.

[1] This work was supported in part by grants from the Committee on Research in Problems of Sex, National Research Council and from the University Committee on Pharmacotherapy.

ance is considered to be between the degree of pituitary inhibition exerted by the thyroid hormone and the quantity of thyroid secretion under the stimulus of thyrotropic hormone. According to this concept, then, an index of the degree of thyroid stimulation is provided by the size of the gland, which may be expected to vary directly with the rate of secretion of the thyrotropic hormone and inversely with the concentration of the circulating thyroid hormone.

With these considerations in mind, it became apparent that information as to the normal rate of secretion of thyroid hormone might be forthcoming from the following general type of experiment. Thyroid hormone formation was prevented by administration of one of the anti-thyroid drugs. Simultaneously, thyroxin, in measured quantities, was administered for an adequate period of time. At the end of this period, the thyroid was removed and weighed. By plotting the weight of the thyroid gland against the quantity of thyroxin administered, the thyroxin requirement for maintenance of the gland at a normal weight, despite the antithyroid drug, was determined. This value, it is considered, is equivalent to the rate of production of thyroid hormone in normal animals maintained under the conditions of the experiment.

In the present paper, the results obtained from experiments of this type are presented.

MATERIAL AND METHODS

Young male rats, weighing 80 to 120 gm. at the beginning of the experiment, were used. Most of the experiments were made in duplicate on two different strains of animals, the Long-Evans strain which had been maintained in this laboratory for several generations, and an albino strain derived from Wistar stock which was purchased from an animal dealer. Only minor differences were detected in the responses of these two strains of animals, and consequently the results have been combined. All animals were fed a stock diet of Purina fox chow. The antithyroid drug selected was 2-thiouracil[2] which was administered *ad libitum* as a 0.1 per cent solution in the drinking water. This assured that the experimental animals received a daily dosage of thiouracil considerably in excess of that required for a maximal histological effect on the thyroid gland (Astwood, 1943).

The various groups of rats were maintained at different temperatures. Cold environments were provided by placing the animals in their usual cages in a cold room which was regulated at 1 ± 1°C., or in a ventilated refrigerator regulated at 5 ± 1°C. Hot environments were secured by maintaining the rats in incubators regulated at 35 ± 1°C., the maximal temperature readily tolerated. Control groups were kept in the animal room, the temperature of which was usually 25° C., but which occasionally varied between 20 and 26.5°C. The temperatures in all cases were recorded by a Taylor recording thermograph.

[2] The thiouracil was supplied by Dr. B. W. Carey, Lederle Laboratories, Inc., Pearl River, New York.

Thyroxin[3] was administered in alkaline saline solutions. In all instances the daily dose was administered hypodermically in a volume of 0.25 cc.

RESULTS

Assay procedure for thyroid hormone. The previous demonstration that the thyroid hyperplasia induced by antithyroid drugs could be inhibited by the concurrent injection of thyroxin or by thyroid feeding, but not by iodide administration (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943) suggested that the degree of inhibition of the hyperplasia might be utilized quantitatively to assay thyroid hormone. In order to test further the specificity of the phenomenon, various doses of *l*-diiodotyrosine[4] and *l*-thyroxin were injected daily during simultaneous administration

TABLE 1. EFFECT OF DIFFERENT DAILY DOSAGES OF DIIDOTYROSINE AND OF THYROXIN UPON THE THYROID WEIGHT OF RATS MAINTAINED AT ROOM TEMPERATURE AND TREATED SIMULTANEOUSLY WITH THIOURACIL FOR 14 DAYS

DIIODOTYROSINE MG./DAY	NO. OF ANIMALS	THYROID WT. MG./100 GM. BODY WEIGHT	THYROXIN μG./DAY	NO. OF ANIMALS	THYROID WT. MG./100 GM. BODY WEIGHT
0.0	4	21.2±0.7	0.0	7	24.1±0.7
0.2	2	19.5±1.2	1.25	8	17.8±0.9
1.0	3	16.9±1.5	2.5	6	12.3±1.5
5.0	3	17.4±1.6	5.0	7	7.6 ±0.9
20.0	3	8.8±1.1	10.0	10	5.3±0.5

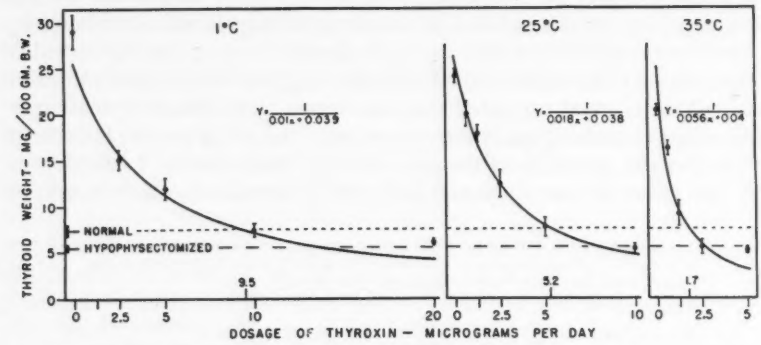
of thiouracil. Table 1 shows the results of these experiments. It is apparent that hyperplasia is effectively prevented by daily administration of about 20 mg. of diiodotyrosine or by about 5 micrograms of thyroxin. Thyroxin, therefore, was about 4,000 times as effective as diiodotyrosine. This value indicates a somewhat lower activity than that found by other methods for the effectiveness of diiodotyrosine. Since the *l*-diiodotyrosine was prepared from thyroid tissue and consequently was not necessarily free from traces of thyroxin and since it has been shown that diiodotyrosine in alkaline solution slowly yields thyroxin (von Muntzenbecker, 1939; Block, 1940; Johnson and Tewkesbury, 1942), it was considered probable that this slight inhibition by diiodotyrosine was caused by the contaminating thyroxin. Consequently, it appeared reasonable to conclude from these experiments that the degree of inhibition of hyperplasia was a function of the amount of administered thyroxin.

Thyroxin requirements at different temperatures. Since it was found that the thyroid hyperplasia induced by treatment with thiouracil is quantitatively inhibited by thyroxin, it became of interest to determine whether the amount of thyroxin necessary to prevent such hyperplasia was the same or different under conditions which might be expected to alter thyroid activity. Consequently, the daily requirement of thyroxin necessary to inhibit thyroid

[3] *l*-Thyroxin, prepared from thyroid tissue, was supplied by Dr. George Harrop, Squibb Institute for Medical Research, New Brunswick, New Jersey.

[4] Eastman Kodak Co., #2129.

enlargement in thiouracil-treated animals was determined for three different environmental temperatures. Thiouracil was administered, and daily injections of thyroxin were given for 14 days. The dosage-response curves determined for these environments are shown in figures 1 and 2. It can readily be



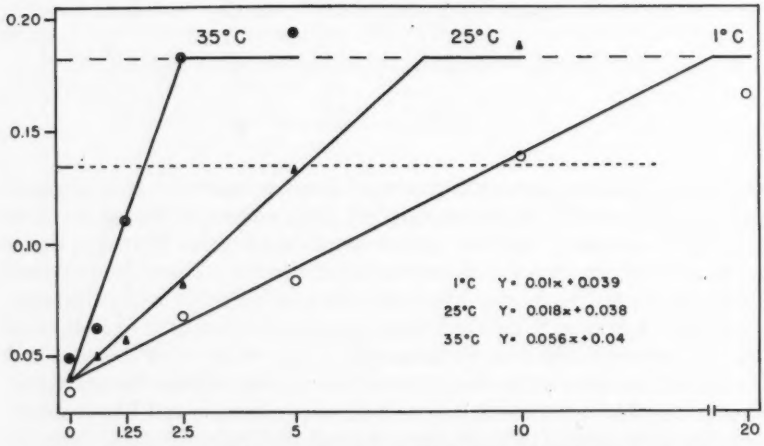


Fig. 1. Thyroid weight of rats maintained at different environmental temperatures and injected daily for 14 days with thyroxin during simultaneous administration of thiouracil. Ninety-three animals were used and distributed about equally among the 13 points. Each point plotted is an average ± its standard error. The thyroid weights and standard errors of 14 normal and 6 hypophysectomized animals are shown by fine and coarse dashes, respectively.

Fig. 2. Dosage response graph of the data shown in figure 1, plotted as daily dose of thyroxin against the reciprocal of thyroid weight in mg./100 gm. of body weight. The coarse and fine dashes represent the thyroid weight in hypophysectomized and normal animals, respectively.

seen that a greater amount of thyroxin was necessary to inhibit hyperplasia in animals maintained in the cold than in the heat. Similarly, the thyroxin requirement of animals maintained at room temperature was intermediate between the requirements at the extremes of temperature.

Rate of development of thiouracil-induced hyperplasia at different temperatures. The previous demonstration that antithyroid drugs interfere with the

formation, but not with the activity, of thyroid hormone leads to the reflection that at the beginning of the treatment there is a store of preformed hormone in the thyroid gland, the release of which delays the onset of thyroid hormone deficiency. Furthermore, as hyperplasia is a compensatory phenomenon, its degree and rate of development should be proportional to the degree of relative thyroid hormone deficiency. Since in the preceding section it was found that the utilization rate of thyroxin is greater at low than at high environmental temperatures, it should follow that hyperplasia after thiouracil would begin sooner in animals maintained in the cold than in those kept in

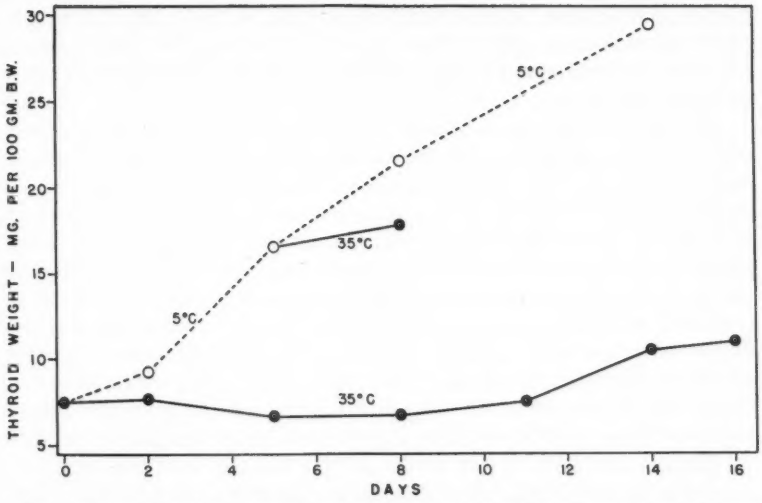


Fig. 3. Thyroid growth rate after administration of thiouracil at different environmental temperatures. Each point represents the average thyroid weight of 3 or more animals. Growth began earlier and proceeded more rapidly at 5°C. than at 35°C. Likewise, after an initial period of 5 days at 5°C., maintenance of a group of rats at 35°C. resulted in a decreased growth rate during the next 3 days.

the hot environment. This possibility was tested by weighing the glands of animals which had been maintained in hot and cold environments and which were killed at intervals after the beginning of thiouracil administration. Figure 3 shows the growth rates of the thyroid gland in the two groups of animals. It is clear that thyroid enlargement began earlier and proceeded at a more rapid rate in the animals maintained in the cold.

It will be noted that the glands of the animals maintained at 35°C. did not enlarge as greatly as did those of the corresponding animals shown in figures 1 and 2. This is considered to be due to the fact that 35°C. is close to the upper limit of temperature tolerated by rats and within this critical range small variations in the environment may be expected to cause wide variations in the response. Furthermore, because of a failure in the ventilating apparatus during this particular experiment, there is reason to suspect that the relative humidity was higher than was the case in the other experiments.

Thyroxin utilization after the onset of hyperplasia. The demonstration of differences in the time of onset of hyperplasia indicates a source of error in the determinations of thyroxin requirements illustrated in figure 1. The source of the error lies in the fact that a significant amount of preformed hormone exists in the thyroid at the beginning of the experimental period. Upon its release from the gland, the effect of this amount of hormone is added to that of the injected thyroxin, and therefore the amount of thyroid hormone required over a given assay period is the sum of the amount of preformed hormone plus the quantity administered. Furthermore, this error, although always in the same direction, would appear to be more significant in the animals that were maintained at high temperatures than in those exposed to a cold environment, since it appears from figure 3 that exhaustion of the preformed hormone occurred sooner in the animals kept in cold temperatures than in a warm environment.

Because of these considerations, the following experiment was devised in order to obtain an estimate of the extent of this error. Animals were maintained on thiouracil at 35°C. for 11 days. On the 11th, 12th and 13th days graded dosages of thyroxin were administed, and on the 14th day the animals were killed. Under these conditions, the amount of thyroxin necessary to prevent a change in the weight of the thyroid was found to be 2.5 micrograms per day, a figure not significantly different from that determined by the experiments in which no opportunity had been provided for exhaustion of the preformed hormone. It appears, therefore, that the magnitude of the error which results from neglecting to take into account the preformed hormone is too small to alter materially the values determined for the thyroid hormone requirements of animals maintained at different environmental temperatures.

Effect of restriction of food intake. During the course of the experiments involving the use of high temperatures, it was noticed that the food intake, growth rate and general activity of the rats were markedly reduced as compared with the same phenomena in animals exposed to lower temperatures. Moreover, it had been found that drugs which produce toxic manifestations in rats cause a decreased thyroid response to antithyroid drugs (Astwood, 1943). It seemed possible, therefore, that the decreased thyroid activity revealed in the high temperature experiments could have been caused by inanition.

The following experiments were undertaken to examine the effect on the thyroid of restriction of food intake. Twenty-seven animals were maintained on thiouracil for 14 days. Nine were kept in the refrigerator, 9 at room temperature, and 9 in the incubator. Three animals in each group of 9 were fed 2.5 gm., 3 were fed 5 gm., and the remaining 3 were fed 10 gm. per day of the stock diet. Table 2 presents the principal results of these procedures. It will be seen that extreme degrees of inanition do indeed inhibit the thyroid hyperplasia which is produced ordinarily by thiouracil. Nevertheless, degrees of starvation sufficient to cause considerable losses in weight still permitted enlargement of the thyroid. Since on an *ad libitum* diet in the hot environment, rats maintain their intial weight or grow slowly, it seems unlikely that the

decreased requirement for thyroxin after such treatment could be explained on a basis of restriction of food intake alone.

Mathematical considerations. Inspection of the dosage-response curves in figure 1 suggests that they are hyperbolic functions. Consequently, the thyroxin dosage was plotted against the reciprocal of the thyroid size. The relationship revealed was approximately linear between the limits of zero dosage and the dosage yielding ordinate values of about 0.18 (fig. 2). Beyond this limit, increasing the dosage caused no further change in the thyroid weight. This phenomenon suggested the possibility that adequate dosages of thyroxin cause a complete suppression of the secretion of thyrotropic hormone. If

TABLE 2. EFFECT OF RESTRICTION OF THE FOOD INTAKE ON THE INCREASE IN WEIGHT OF THE THYROID GLAND OF RATS TREATED WITH THIOURACIL FOR 14 DAYS

TEMP. °C.	NO. STARTED	NO. SURVIVING	BODY WEIGHT CHANGE %	CHANGE IN TW/BW %
		2.5 gm. of food per day		
35	3	1	−36	−11
25	3	0		
1	3	0		
		5 gm. of food per day		
35	3	3	+ 2	34
25	3	2	−11	78
1	3	0		
		10 gm. of food per day		
35	3	1	+35	160
25	3	3	+54	108
1	3	0		

this were true, injections of larger amounts could cause no further effect upon the resulting size of the thyroid gland. Moreover, the maximum degree of inhibition produced by thyroxin should, then, be identical with that produced by hypophysectomy. These considerations prompted a comparison of the size of the thyroid in animals which had been hypophysectomized for 14 days with that of thiouracil-treated animals in which the thyroid had been inhibited by thyroxin for a similar period of time. The results are presented in figures 1 and 2 and show an equal reduction in thyroid weight with either procedure. Consequently, it can be stated that an approximately linear relationship exists between thyroid dosage and the reciprocal of the thyroid-weight, between the limits of zero pituitary stimulation and the maximal pituitary stimulation obtainable at any given temperature.

The advantage gained by plotting the data in the fashion shown in figure 2 lies in the fact that the resulting graphs are linear functions, and, as such, allow the utilization of the entire body of data for the determination of their descriptive formulae. These formulae, which are indicated in the figures, have been determined by the method of least squares. The characteristics of the lines are described by the slope and by the intercept value. It can be seen that as the environmental temperature increases the slope increases. Since

the slope describes the rate of change of the thyroid weight as a function of the thyroxin dosage, it can be stated that at low environmental temperatures more thyroxin is required to produce an equivalent inhibition of thyrotropin secretion than at higher temperatures. Furthermore, since it is demonstrated in figure 3 that the growth rate of the thyroid is greater in cold than in hot environments, the degree of thyrotropic stimulation is inversely proportional to the environmental temperature.

The amount of thyroxin required to maintain the thyroid at any given weight may be calculated from the formulae derived above, or may be obtained graphically by determining the intersection of the lines in figure 2 with a horizontal projection at the appropriate value on the y axis. The

TABLE 3. CHANGES IN THE BASAL METABOLIC RATE OF RATS TREATED WITH THIOURACIL AND THYROXIN

TREATMENT	NO.	METABOLIC RATE (CAL./SQ.M./HR.)	CHANGE FROM NORMAL %
Normal controls	6	37.1±0.4	0.0
Thiouracil 9 to 12 days	4	31.2±1.0	−16.0
2 to 3 mo.	4	17.1±0.1	−53.0
Thiouracil 9 to 12 days Thyroxin, daily			
1.25 μg.	1	31.2	−16.0
2.5 μg.	1	35.1	− 4.6
5.0 μg.	1	35.1	− 4.6
10.0 μg.	1	44.0	+19.0

values found to maintain the thyroid at a normal weight are indicated. Approximately 9.5 micrograms of thyroxin are required at 1°C., 5.2 micrograms at room temperature, and 1.7 micrograms at 35°C.

Metabolic rate at different levels of thyroid activity. Because of technical difficulties too great reliance can not be placed on measurements of metabolic rates in rats. Nevertheless, the thyroxin requirements as revealed by metabolic rates and those determined by the prevention of thyroid hyperplasia during the administration of antithyroid drugs should agree roughly. Moreover, the magnitude of the effect of thiouracil on the metabolic rate should give some indication of the completeness of the functional thyroidectomy which the drug produces. For these reasons, the oxygen consumption of animals at different states of thyroid activity was determined.

The metabolic rate, measured under conditions as near basal as possible, was markedly influenced by thiouracil and thyroxin. Table 3 shows that administration of thiouracil for 9 to 12 days was followed by a rate of −16 per cent, while prolonged administration caused a further fall to −53 per cent. This decrease in metabolic rate was prevented by the simultaneous administration of thyroxin. Judged by the metabolic rate, the daily thyroxin requirement was between 5 and 10 micrograms per day, a figure in substantial agreement with that determined by the prevention of thiouracil-induced hyperplasia.

DISCUSSION

Quantitative determinations of the biologic activity of thyroid hormone by methods heretofore available have not permitted a degree of precision that is desirable for accurate work. The commonly used methods, *e.g.*, those dependent upon amphibian metamorphosis, calorigenic effects and the reversal of myxedematous changes in animals and human beings, while technically difficult, are not correspondingly precise. An examination of the standard errors shown in figure 1 revels that the method developed for the studies reported here is subject to little variation. Moreover, the application of this technique to the assay of relatively small amounts of thyroid hormone is attended by even less error than that indicated in figure 1 which includes data accumulated over a period of several months. For assay purposes greater accuracy would result from the simultaneous determination of known and unknown samples under identical environmental conditions.

From the data presented in the preceding sections it may be concluded that the rate of secretion of the thyroid hormone is approximately doubled by exposure to cold and is decreased to a third of its normal value by exposure to heat. This conclusion is based upon two assumptions, namely, that thiouracil completely inhibits the formation of thyroxin, and that the restoration or maintenance of the normal thyroid weight by administration of thyroxin to thiouracil-treated animals is a valid measure of the normal rate of secretion of the gland.

Concerning the first assumption, it can only be said that the available evidence indicates that the amount of thyroid hormone produced during thiouracil treatment must be vanishingly small. In support of this statement is the pronounced and progressive hyperplasia of the thyroid despite the fact that thyroxin in extremely small amounts inhibits this hyperplasia. Furthermore, it has been found that growth can be inhibited and that cretinous animals are produced when the drug is administered to very young animals (unpublished data). The development of cretins supports the belief that thyroid hormone is completely inhibited, as it has been shown that thyroidectomy must be performed very early in life to secure this effect in rats (Salmon, 1936). Lastly, the metabolic studies presented in the section dealing with metabolic rate at different levels of thyroid activity have revealed resting oxygen consumption rates as low as −53 per cent of normal, a figure actually lower than that which has been reported for surgically thyroidectomized rats. This observation may find explanation in the recent observations of Chapman (1941) and of Morton, Chaikoff, Reinhardt, and Anderson (1943) that small amounts of thyroxin can be formed even in the complete absence of the thyroid gland.

The second assumption, that the amount of thyroxin necessary to maintain normal thyroid weight in thiouracil-treated animals is a valid index of the rate of secretion of thyroid hormone appears reasonable on the basis of the known facts concerning pituitary-thyroid relationships. It is well known that the secretion of thyrotropic hormone increases after thyroidectomy and that thyrotropin secretion is decreased by thyroid treatment. From consid-

eration of the proof presented elsewhere that thyroid hormone formation is inhibited by antithyroid drugs, it would appear that the endocrinological events following administration of the drug should be equivalent to those following thyroidectomy. The only differences consist in the facts that thyroid tissue is present in the treated animal, and that this tissue retains its capacity for growth under the stimulus of the thyrotropic hormone. Consequently, an alternative explanation for the phenomena described above would require evidence other than that which has thus far been adduced.

The demonstration that cold causes an increased production of thyroid hormone raises the problem of the utilization of the substance. Presumably an increased rate of secretion of thyroid hormone could be followed either by a rise in its concentration in the blood and tissue or by an increased rate of destruction and excretion, or by both. Evidence supporting the contention that there is a higher concentration of thyroid hormone comes from the demonstration that the basal metabolic rate is elevated during exposure to cold (Ring, 1939) and from the fact that an increase in blood organic iodine accompanies elevations of the metabolic rate (Salter, 1940). On the other hand, the possibility that the rate of utilization of thyroid hormone is increased by cold is by no means excluded. Indeed, it is not unlikely that the rate of utilization is proportional to the concentration in the organism.

SUMMARY

The maintenance or restoration of normal thyroid weight by the administration of thyroxin to rats simultaneously treated with the antithyroid drug, thiouracil, has been used as the basis of an assay procedure for thyroid hormone. The rate of thyroid enlargement in response to thiouracil administration was low in hot environments and high when rats were maintained in the cold. A quantity of thyroid hormone equivalent to 5.2 micrograms of thyroxin daily was required to maintain a thyroid of normal weight in young male rats kept at room temperatures averaging 25°C. At 1°C. the thyroxin requirement was increased to 9.5 micrograms, while at 35°C. it was decreased to 1.7 micrograms. These values are considered to be quantitatively equivalent to the amount of hormone produced by the normal thyroid gland under these conditions.

REFERENCES

ASTWOOD, E. B.: *J. Pharmacol. and Exper. Therap.* 78: 79. 1943.
ASTWOOD, E. B., J. SULLIVAN, A. BISSELL AND R. TYSLOWITZ: *Endocrinology* 32: 210. 1943.
BLOCK, P.: *J. Biol. Chem.* 135: 51. 1940.
CHAPMAN, A.: *Endocrinology* 29: 686. 1941.
CRAMER, W.: Fever, Heat Regulation, Climate and the Thyroid-Adrenal Apparatus. Longmans, Green and Co., London, 1928.
JOHNSON, T. B., AND L. B. TEWKESBURY: *Proc. Nat. Acad. Sci.* 28: 73. 1942.
MACGREGOR, R. G. S., AND G. L. LOH. *J. Physiol.* 99: 496. 1941.
MACKENZIE, C. G., AND J. B. MACKENZIE: *Endocrinology* 32: 188. 1943.
MORTON, M. E., I. L. CHAIKOFF, W. O. REINHARDT AND E. ANDERSON: *J. Biol. Chem.* 147: 757. 1943.
PETERSEN, W. F.: The Patient and the Weather. Edwards Bros., Inc., Ann Arbor, Michigan. 1937.
RING, G. C.: *Am. J. Physiol.* 137: 582. 1939.
SALMON, T. N.: *Proc. Soc. Exper. Biol. & Med.* 27: 101. 1936.
SALTER, W. T.: The Endocrine Function of Iodine. Harvard University Press, Cambridge, Mass. 1940.
VON MUNTZENBECKER, P.: *Zeit. physiol. Chem.* 261: 253. 1939.

NEW BOOKS

HUMAN EMBRYOLOGY. Joseph Krafka, Jr., M.D., Ph.D. Professor of Microscopic Anatomy, University of Georgia School of Medicine. Composition binding. 222 illustrations; 395 pages. $4.75. New York, Paul B. Hoeber, Inc. 1942.

THIS VOLUME is one of the Medical Student Series, "presenting what the student has time to read, at a price he can afford to pay." In its 400 comparatively small and well-leaded pages is condensed the organogeny of the human body, told as simply as seems possible and illustrated by 222 drawings, all made in a uniform style by the author himself, some original, the majority redrawn from published works. On the whole, the description of the well-selected stages, with the explanatory diagrams, should give the student a clear view of the essential processes involved and lay the basis for an intelligent understanding of anatomy and surgical procedures. Such is, of course, one of the chief arguments for the study of human embryology in the medical curriculum.

The author states that he has effected curtailment of space by leaving out all references to comparative embryology, to physiology of development and to laboratory directions. This resolution was carried through but not without occasional lapses. Thus the author could not resist comparing the pronephros with the nephridium of the earthworm (p. 218). Speculations on the origin of teratomata, parasitic "twins" or polyembryony from parthenogenetic ovarian ova (p. 24), after Loeb's theory, or from dislodged and vagrant blastomeres of the embryonic vesicles (p. 60) or again from splitting of Hensen's node (p. 75) may serve to whet the student's appetite for theorizing but hardly simplify the text. Gastrulation is of necessity inadequately treated, inasmuch as the modern viewpoint is based entirely on the experimental method, which is beyond the scope of the book; hence the section on Theoretical Considerations of Axiogenesis (p. 74) might well have been omitted. To cite one more example, it would have been well to have sidestepped the causal factors in the development of flexure in the heart (p. 115), for the mechanical explanation stated has been shown to be wrong, inasmuch as the flexures occur also in isolated heart in tissue cultures.

It is, of course, true that, in any short treatise like the one before us, unfortunately, statements have to be made more or less categorically.

The results of the author's revamping of time-honored illustrations are not always happy. Some are, indeed, mere caricatures of the originals, others quite meaningless. What kind of a notion, for example, will a student carry away concerning the all-important spiral arteries of the endometrium by a study of figure 9?

An adequate list of references is appended to each chapter. But these were apparently turned over to the tender mercies of a uni-lingual secretary, for there is scarcely a German reference that does not have from one to four errors. The printer was especially chary with the German umlaut; but an extra diaeresis was (wrongly) added to Gartner—except once, namely, in figure 133 where the name of the discoverer of remnants of the Wolffian duct in the female organism is correctly spelled.

Passing to the chapters on the male and female reproductive organs and the hormonal control of the genital system, the reviewer has the feeling that these should have been omitted, the first two because too brief to be of any value, the latter for the double reason of excessive brevity and confusing presentation. The editor of the series of which the present volume is one, in stressing the need of clarity in student texts, could not possibly have been thinking of these chapters.

Witness, for example, the variety of terms used for the same hormone. Thus progestin, progesterone, corpus luteum hormone have all been employed, when the reader is suddenly

confronted with corporin. But the extreme test of the student's gift of riddle-guessing is found on page 45 where, for follicle-stimulating hormone (F.S.H.) and luteinizing hormone of the pituitary (L.H.), terms now in general use, the author brings in Prolan A and Prolan B, Rho 1 and Rho 2 (on another page A.P.R. 1 and A.P.R. 2) as well as the trade-name Antuitrin S! The fault of the author's confusion should, however, the reviewer believes, be partly laid at the door of the endocrinologist, whose nomenclature has not yet been stabilized.

Misstatements also abound, as for example, that the guinea pig does not need corpora lutea after the third day of pregnancy (p. 43); that steroidal hormones are injected in a vehicle of alcohol or acetone (p. 44). On the subject of the time of ovulation the author cites outdated material now only of historical interest, as *e.g.*, Zangemeister's "clinical" data, figure 11. Latz's old data are quoted (p. 54) but not his very extensive new series of cases. On page 32 the ovum is categorically stated to make the tubal journey in 3 to 6 days (on what authority?); but on page 28 and again on page 57 the author refers to an object, estimated at 7 days of age, washed from an oviduct and alleged to be a human ovum in the morula stage.

One wonders if it would not be well for authors of embryological texts to invite their colleagues to contribute the chapters on genetics and endocrinology, should these subjects be deemed essential to an understanding of embryology.

CARL G. HARTMAN

AUTHOR INDEX, VOLUME 32

ANDREWS, F. N., AND R. E. ERB. Ascorbic acid gonadotropic hormone relationships in the chick, 140

ASTWOOD, E. B., J. SULLIVAN, ADELE BISSELL AND R. TYSLOWITZ. Action of certain sulfanomides and of thiourea upon the function of the thyroid gland of the rat, 210

——, see Dempsey, E. W., 509

BARNES, R. H., see Szego, Clara M., 367

BATES, R. W., see Lahr, E. L., 251

BENJAMIN, J. A., see Krichesky, B., 345

BERGMAN, A. J., AND C. W. TURNER. Effect of pregnancy and lactation upon the thyrotropic hormone of the rabbit, 59

——, see Mixner, J. P., 298

BISCHOFF, F. Reaction of chorionic gonadotropin with phenylisocyanate, 260

BISKIND, M. S., AND G. R. BISKIND. Inactivation of testosterone propionate in the liver during vitamin B complex deficiency. Alteration of the estrogen-androgen equilibrium, 97

BISSELL, ADELE, see Astwood, E. B., 210

BLANCHARD, E. W., A. H. STUART AND R. C. TALLMAN. Studies on a new series of synthetic estrogenic substances, 307

Book reviews. Die thrombozyten des menschlichen Blutes and Ihre Beziehung zum Gerinnungs- und Thrombosevorgang. A. Fonio, 371

Human Embryology. Krafka, J. Jr., 519

Pediatric Gynecology. Goodrich C. Schauffler, 451

The Adrenal Cortex in Adaptation to Altitude, Climate and Cancer. Edward S. Sundstroem and George Michaels, 452

Sex Hormones. Biological symposia, vol. IX. Edited by F. C. Koch, 370

BOYD, E. M., B. K. LEE AND MARY E. T. STEVENS. Effect of optical stimuli on output of urine in albino rats, 27

BUCHER, N. L. R., see Seegar Jones, G. E., 46

BUTCHER, E. O. Oxygen consumption of skin and hair growth after adrenalectomy in the white rat, 493

CANTAROW, A., A. E. RAKOFF, K. E. PASCHKIS, L. P. HANSEN AND A. A. WALKLING. Excretion of estrogen in the bile, 368

——, see Paschkis, K. E., 41

CHAIKOFF, I. L., see Kaplan, A., 247

CHAMBERS, R., see Hyman, C., 310

CLARKE, A. P. W., see Cleghorn, R. A., 170

CLEGHORN, R. A. A comparative assay of desoxycorticosterone acetate and acetoxypregnenolone in the adrenalectomized dog, 165

——, A. P. W. CLARKE AND W. F. GREENWOOD. Activity of desoxycorticosterone acetate in propylene glycol by oral and intravenous routes in adrenalectomized dogs, and its effect on the cardiac arhythmia of adrenal insufficiency, 170

DEMPSEY, E. W., AND E. B. ASTWOOD. Determination of the rate of thyroid hormone secretion at various environmental temperatures, 509

——, AND H. F. SEARLES. Environmental modification of certain endocrine phenomena, 119

DOHAN, F. C., see Lukens, F. D. W., 475

DRILL, V. A., R. OVERMAN AND J. H. LEATHEM. Relation of the B vitamins to ovarian function during experimental hyperthyroidism, 327

EMERY, F. E., C. S. MATTHEWS AND F. L. TABRAH. Circulatory effects of diethylstilbestrol in cats and rats with a note on intestinal absorption, 77

ENGEL, F. L., see Tepperman, J., 373; 403

ENTENMAN, C., see Kaplan, A., 247

ERB, R. E., see Andrews, F. N., 140

ETTINGER, G. H., AND D. JEFFS. Effects in the dog of infusion of adrenal cortical extract on exercise tolerance, blood constituents and adrenal cortex, 351
EVANS, G. T., see Samuels, L. T., 422
EVANS, H. M., MIRIAM E. SIMPSON, W. MARX AND E. KIBRICK. Bioassay of the pituitary growth hormone. Width of the proximal epiphysial cartilage of the tibia in hypophysectomized rats, 13
——, see Gorbman, A., 113
EVERETT, J. W. Further studies on the relationship of progesterone to ovulation and luteinization in the persistent-estrous rat, 285
EVERSOLE, W. J., AND R. GAUNT. Methods of administering desoxycorticosterone and the problem of its inactivation by the liver, 51

FERGUSON, J. Effect of propylene glycol and progesterone on motility of the isolated intestine of the rabbit, 57
FERRILL, H. W. Effect of chronic insulin injections on reproduction in white rats, 443
FLEISCHMANN, W., H. B. SHUMACKER AND W. L. STRAUS, JR. Influence of age on the effect of thyroidectomy in the rhesus monkey, 238
FORBES, T. R. Additional data on the relative absorption rates of subcutaneous pellets of various crystalline compounds in the rat, 282
FORD, Z. W., JR., see Sure, B., 433

GARDNER, W. U. Influence of sex and sex hormones on the breaking strength of bones of mice, 149
——, see Treadwell, A. deG., 161
GAUNT R. See Eversole, W. J., 51
GINTHER, G. B., see Ingle, D. J., 410
GORBMAN, A., AND H. M. EVANS. Beginning of function in the thyroid of the fetal rat, 113
GRAEF, I., see Ralli, Elaine P., 1
GREENWOOD, W. F., see Cleghorn, R. A., 170
GREEP, R. O., see Hellbaum, A. A., 33
GROAT, R. A., Adrenocortical-like tissue in the ovaries of the adrenalectomized ground squirrel (*Citellus tridecemlineatus*), 488

HANSEN, L. P., see Cantarow, K. E., 368
HARTMAN, F. A., D. E. SMITH AND LENA A. LEWIS. Adrenal functions in the opossum, 340
——, see Smith, D. E., 145; 437
HECHTER, O. Effect of histamine upon capillary permeability in the skin and muscle of normal and adrenalectomized rats, 135
HELLBAUM, A. A., AND R. O. GREEP. Qualitative changes induced in gonadotropic complex of pituitary by testosterone propionate, 33
HELLER, C. G., AND EMILY J. HELLER. Metabolism of estrogens: effect of pregnancy upon metabolism *in vitro* of estrone, estradiol and estriol, 64
HELLER, EMILY J., see Heller, C. G., 64
HILL, B. R., AND B. B. LONGWELL. Urinary steroids from breast cancer patients, 319
HOOKER, C. W., AND C. A. PFEIFFER. Effects of sex hormones upon body growth, skin, hair, and sebaceous glands in the rat, 69
HUNT, T. E. Mitotic activity in the anterior hypophysis of female rats of different age groups and at different periods of the day, 334
HYMAN, C., AND R. CHAMBERS. Effect of adrenal cortical compounds on edema formation of frogs' hind limbs, 310

INGLE, D. J., G. B. GINTHER AND J. NEZAMIS. Effect of diet in rats on adrenal weights and on survival following adrenalectomy, 410

JEFFS, D., see Ettinger, G. H., 351
JONES, D. G., AND W. F. LAMOREUX. Estimation of the size of comb on live fowl, 356

KAPLAN, A., C. ENTENMAN AND I. L. CHAIKOFF. Effects of insulin on the blood lipids of man, 247
KENDALL, E. C., see Reinecke, R. M., 505
KIBRICK, E., see Evans, H. M., 13
KRICHESKY, B., J. A. BENJAMIN AND C. SLATER. Inactivation of endogenous androgens by the liver in rabbits, 345

LAHR, E. L., R. W. BATES AND O. RIDDLE. Non-specific results obtained with

micro-method for assay of prolactin, 251
LAMOREUX, W. F. Effect of differences in light and temperature upon the size of combs on White Leghorns, 497
——, see Jones, D. G., 356
LAQUEUR, G. L. Effects of testosterone propionate on the mammary glands of female albino rats, 81
LAWRENCE, J. H., see Treadwell, A. deG., 161
LEATHEM, J. H., see Drill, V. A., 327
LEE, B. K., see Boyd, E. M., 27
LEIN, A. Studies on the fixation of radioactive iodine by the rabbit thyroid, 429
LEONARD, S. L. Stimulation of mammary glands in hypophysectomized rats by estrogen and testosterone, 229
LEVY-HOCHMAN, SH., see Sulman, F., 293
LEWIS, LENA A., see Hartman, F. A., 340; Smith, D. E., 437
LONG, C. N. H., see Tepperman, J., 373; 405
LONGWELL, B. B., see Hill, B. R., 319
LUKENS, F. D. W., F. C. DOHAN AND M. W. WOLCOTT. Pituitary diabetes in the cat: recovery following phlorhizin treatment, 475

MACKENZIE, C. G., AND J. B. MACKENZIE. Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism, 185
MACKENZIE, J. B., see MacKenzie, C. G., 185
MCKELVEY, J. L., see Samuels, L. T., 422
MARVIN, H. N., AND R. K. MEYER. Progonadotropic and aspecific effects of the serum of a horse immunized with extracts of sheep pituitary glands, 271
——, AND GUINEVERE C. SMITH. Technique for thyroidectomy in the pigeon and the early effect of thyroid removal on heat production, 87
MARX, W., see Evans, H. M., 13
MATTHEWS, C. A., see Emery, F. E., 77
MEYER, R. K., see Marvin, H. N., 271
MILLER, H. C. Effect of the injection of sex hormones on serum and muscle electrolyte of rats, 443
MILLER, R. A., AND O. RIDDLE. Ability of adrenal cortical hormones, prolactin and thyroxin to sustain weight of body and viscera of hypophysectomized pigeons, 463
MIXNER, J. P., A. J. BERGMAN AND C. W. TURNER. Relation of certain endocrine glands to body weight in growing and mature guinea pigs, 298
MOORE, C. R., AND C. F. MORGAN. First response of developing opossum gonads to equine gonadotropic treatment, 17
MORGAN, C. F., see Moore, C. R., 17

NEZAMIS, J., see Ingle, D. J., 410

OVERMAN, R., see Drill, V. A., 327

PASCHKIS, K. E., AND A. CANTAROW. Sugar content of blood, lymph and artificial peritoneal fluid following injection of insulin, 41
——, see Cantarow, A., 368
PFEIFFER, C. A., see Hooker, C. W., 69
PINCUS, G. New color reaction for certain urinary 17-ketosteroids, 176
PRATT, P. C. Evidence against a progesterone-like action of ascorbic acid, 92

RAAB, W. Corrected evaluation of the results obtained with Shaw's colorimetric 'adrenalin' method, 226
RADCLIFFE, C. E. Observations on the relationship of the thyroid to the polyuria of experimental diabetes insipidus, 415
RAKOFF, A. E., see Cantarow, A., 368
RALLI, ELAINE P., AND I. GRAEF. Stimulating effect of adrenalectomy on hair growth and melanin deposition in black rats fed diets adequate and deficient in the filtrate factors of vitamin B., 1
REFORZO-MEMBRIVES, J. Thyroid-inhibiting action of the hypophyses of rats fed with thyroid, 263
REINECKE, R. M., AND E. C. KENDALL. A comparison of the influence of some crystalline hormones of the adrenal cortex on the deposition of glycogen in the liver, 505
REMINGTON, J. W. Effect of adrenal cortical extract and certain steroid hormones on the response of adrenal-

ectomized rats to intraperitoneal injections of an isotonic glucose solution, 129
RIDDLE, O., see Lahr, E. L., 251; Miller, R. A., 463

SAMUELS, L. T., G. T. EVANS AND J. L. MCKELVEY. Ovarian and placental function in Addison's disease, 422
SEARLES, H. F., see Dempsey, E. W., 119
SEEGAR JONES, G. E., AND N. L. R. BUCHER. Study of excretion of pituitary hormones in human urine, 46
SELYE, H. Production of testis atrophy by steroids, 116
——, Rôle of bile in absorption of steroids, 279
SHUMACKER, H. B., see Fleischmann, W., 238
SIMPSON, MIRIAM E., see Evans, H. M., 13
SKLOW, J. Prolongation of sex hormone effects by adsorption on powdered carbon, 109
SLATER, C., see Krichesky, B., 345
SMITH, D. E., AND F. A. HARTMAN. Influence of adrenal preparations on fish melanophores, 145
——, LENA A. LEWIS AND F. A. HARTMAN. Sodium retention in the opossum, 437
——, see Hartman, F. A., 340
SMITH, GUINEVERE C., see Marvin, H. N., 87
STEVENS, MARY E. T., see Boyd, E. M., 27
STUART, A. H., see Blanchard, E. W., 307
STRAUS, W. L., JR., see Fleischmann, W., 238
SULLIVAN, J., see Astwood, E. B., 210
SULMAN, F., SH. LEVY-HOCHMAN AND H. G. TIETZ. Selective bismuth melanosis of the female genital tract induced by treatment with sex hormones, 293
SURE, B., AND Z. W. FORD, JR. Influence of hyperthyroidism on urinary excretion of thiamin and riboflavin, 433
SZEGO, CLARA M., AND R. H. BARNES. Dietary fatty livers in mice and sensitivity to exogenous estrogen, 367

TABRAH, F. L., see Emery, F. E., 77
TALLMAN, R. C., see Blanchard, E. W., 307
TEPPERMAN, J., F. L. ENGEL AND C. N. H. LONG. A review of adrenal cortical hypertrophy, 373
——, ——, ——. Effect of high protein diets on size and activity of the adrenal cortex in the albino rat, 403
TIETZ, H. G., see Sulman, F., 293
TREADWELL, A. DEG., W. U. GARDNER AND J. H. LAWRENCE. Effect of combining estrogen with lethal doses of roentgen-ray in Swiss mice, 161
TURNER, C. W., see Bergman, A. J., 59; Mixner, J. P., 298
TYSLOWITZ, R. Effect of hypophysectomy on the concentration of ascorbic acid in the adrenals, 103
——, see Astwood, E. B., 210

VAICHULIS, J. A. Effect of autolysis on the differential solubility of the principles of the posterior lobe of the pituitary body, 361

WALKLING, A. A., see Cantarow, A., 368
WELLS, L. J. Effects of large doses of androgen on the testis in the ground squirrel, *Citellus tridecemlineatus*, 455
WOLCOTT, M. W., see Lukens, F. D. W., 475

SUBJECT INDEX, VOLUME 32

Addison's disease,
  ovarian function in, 422
  placental function in, 422
Adrenal, weight/body weight ratio in guinea pig, 298
Adrenal cortex, ascorbic acid content, 103
  hypertrophy, review, 373
  response to diet, 403, 410
Adrenal cortical hormones, and electrolyte balance, 129, 310, 351, 437
  effect on adrenal cortex, 351
    blood sugar, 351
    exercise tolerance, 351
  crystalline, 505
  extract, effects in hypophysectomized pigeons, 463
  sodium factor fraction, effect on electrolyte metabolism, 437
  synthetic
    acetoxypregnenolone
      maintenance after adrenalectomy, 165
    desoxycorticosterone
      pellet absorption, 282
    desoxycorticosterone acetate
      bile and enteral absorption, 279
      effect in hypophysectomized pigeons, 463
      electrolyte transfer, 129, 437
      inactivation by liver, 51
      maintenance in adrenalectomy, 51, 165, 170
Adrenalectomy, acetoxypregnenolone, 165
  adrenocortical-like tissue in ovaries, 488
  blood glucose following, 340
  capillary permeability and histamine, 135
  desoxycorticosterone acetate in, 51, 165, 170
  electrolyte transfer in, 129, 340
  hair growth, 1, 493
  oxygen consumption of skin, 493
  survival in opossum, 340
  vitamin B filtrate factors, 1
Adrenalin, Shaw's colorimetric method, 226
Adsorption and prolonged hormone action, 109
Androgen-estrogen equilibrium and testis atrophy, 116
  vitamin B, 97
Androgens, excretion in cases of breast cancer, 319
  inactivation by the liver, 345
  pellet absorption, 282
  prolongation of action by adsorption, 109
  androsterone, excretion in cases of breast cancer, 319
  17-ketosteroids, excretion in Addison's disease, 422
  testosterone, bile and enteral absorption, 279
    effect on testis, 455
  testosterone propionate, breaking strength of bone, 140
    effect on growth, 69
      hypophyseal gonadotropins, 33
      mammary growth, 81, 229
      organ weights, 69
      testis, 455
    electrolyte transfer 129, 443
    inactivation in liver, 97
Ascorbic acid, content of adrenal cortex, 103
Assay, acetoxypregnenolone, 165
  desoxycorticosterone acetate, 165
  hypophyseal growth factor, 13
  prolactin, 251
  thyroid hormone, 509
Association Notice, 118
Augmentation phenomena, gonadotropin and vitamin C, 140

Bile and enteral absorption, 279
  estrogen excretion in, 279
Bismuth melanosis, 293
Bone, response to sex hormones, 149
Book reviews, 305, 370, 371, 451, 452, 519

Capillary permeability, and adrenalectomy, 135
Chorionic gonadotropins, adsorption, 109
  inactivation, 260

Comb size, effect of light and temperature, 497
estimation in White Leghorns, 351

Desoxycorticosterone acetate, see adrenal cortical hormones, synthetic
Diabetes, pituitary, recovery in cats, 475
Diabetes insipidus, relation to thyroid feeding, 415
Diet, response of adrenal cortex to, 403, 410
Diethylstilbestrol, see estrogens
Diuresis and light stimulus, 27

Endocrine gland/body weight ratio in guinea pig, 298
Endocrine-vitamin relationships
adrenals and vitamin B_1, 1
vitamin C, 103
androgens and vitamin B, 97
gonadotropins and vitamin C, 140
progesterone and vitamin C, 92
thyroid and vitamin B, 227, 433
Equine gonadotropins, action with ascorbic acid, 140
gonad response in opossum, 17
Estradiol compounds, see estrogens
Estrogen-androgen ratio, 97, 116
Estrogens, activity in presence of fatty livers, 367
excretion in Addison's disease, 422
in bile, 368
pellet absorption, 282
diethylstilbestrol
adsorption studies, 109
effect on circulatory system, 77
estradiol, metabolism *in vitro*, 64
estradiol benzoate
and muscle electrolytes, 443
action with roentgen-ray, 161
breaking strength of bones, 140
effect on growth, 69
organ weight, 69
electrolyte transfer and adrenalectomy, 129
estradiol dipropionate
and muscle electrolyte metabolism, 443
mammary growth, 229
estriol, metabolism *in vitro*, 64
estrone, metabolism *in vitro*, 64
new synthetic series, 307
Estrous cycle, and hyperthyroidism, 327
following section of hypophyseal stalk, 119

Exercise tolerance and adrenal cortical hormones, 351

Growth, effect of sex hormones on, 69
sulfonamides and thioureas on, 185

Hair growth, and adrenalectomy, 1, 493
sex hormones, 69
vitamin B_1, 1
Histamine, capillary permeability after adrenalectomy, 135
Hypophyseal hormones, production after section of stalk, 119
corticotropin
effect in hypophysectomized pigeons, 463
gonadotropins, see also chorionic gonadotropins and equine gonadotropins
antisera, 271
augmentation and inhibition, 271
variation in content after androgens, 33
follicle stimulating factor, in human urine, 46
level after androgens, 27
luteinizing factor
level after androgens, 27
menopause urine
mammary growth, 229
pituitary
effect in hypophysectomized pigeons, 463
growth factor
assay, 13
posterior lobe
rôle in diuresis, 27
prolactin, assay, 251
effect in hypophysectomized pigeons, 463
thyrotropin
effect in hypophysectomized pigeons, 463
of thyroid on level, 263
hypophyseal content during lactation, 59
in pregnancy, 59
Hypophysectomy, ascorbic acid content of adrenal, 103
assay, growth factor, 13
maintenance of pigeons after, 463
mammary growth in males, 229
response in, to sulfonamides and thioureas, 185
Hypophysis, mitosis in rat, 334

posterior lobe, hormone content after incubation, 361
response to sulfonamides and thioureas 185
studies following section of stalk, 119
weight/body weight ratio, guinea pig, 298

Insulin, absorption studies, 109
blood lipids in man, 247
effect on reproduction, 439
sugar level in body fluids, 41
Iodine, fixation by thyroid, 429

Ketosteroids, color reactions, 174

Light stimulus and diuresis, 27
Liver, and estrogen activity, 367
inactivation, desoxycorticosterone acetate, 51
testosterone propionate, 97

Mammary gland, growth in hypophysectomized males, 229
response to androgens in females, 81
Metabolism
carbohydrate, blood glucose after adrenalectomy, 340
blood sugar and adrenal cortical hormones, 351
electrolyte, and adrenal cortical hormones, 129, 310, 340, 351, 437
and androgens, 129, 443
in diabetes insipidus, 415
estrogen, in vitro, 64
nitrogen, in diabetes insipidus, 415
oxygen, of skin, 493

Ovary, adrenocortical-like tissue, 422
function in Addison's disease, 422
response to equine gonadotropins, 17
weight/body weight ratio in guinea pig, 298
Ovulation, and section of pituitary stalk, 119
induction in persistent estrus, 285

Pancreas, islet structure in pituitary diabetes, 471
Pellet implants, crystalline hormones, absorption rates, 282
Persistent estrous rats, 285
Phlorhizin, and recovery of pituitary diabetes, 471
Placenta, function in Addison's disease, 422
Pregnancy, estrogen metabolism, 64
Pregnanediol, excretion in Addison's disease, 422
excretion in cases of breast cancer, 319
Progesterone, action in persistent-estrous rats, 285
and uterine motility *in vitro*, 51
electrolyte transfer and adrenalectomy, 129
enteral absorption and bile, 279
pellet absorption, 282
prolonged action by adsorption, 109
Prostate, ocular implants, rabbit, 345
Pseudopregnancy, induction after section of hypophyseal stalk, 119

17-ketosteroids, see androgens
Shaw's colorimetric adrenalin method, corrected evaluation, 226
Skin, oxygen consumption of, 493
Stilbestrol, see estrogens
Sulfonamides and the thyroid, 185, 210

Testis, atrophy induction, 116
effect of androgen on, 455
response to equine gonadotropin, 17
gonadotropin and ascorbic acid, 140
transplants, 345
weight/body weight ratio in guinea-pig, 298
Testosterone compounds, see androgens
Thioureas and thyroid gland, 185, 210
Thyroid, fetal function, rat, 113
fixation of radioactive iodine, 429
hyperthyroidism and vitamin B complex, 433
and ovarian function, 327
radio-active iodine, 113, 429
rate of hormone secretion, 509
relationship to experimental diabetes insipidus, 415
response to sulfonamides and thioureas 185, 210
weight/body weight ratio in guinea-pig, 298
Thyroidectomy, basal heat production following, in pigeons, 87
chloride metabolism following, 415
effect in monkey, 238
technique in pigeon, 87

Uterus, motility studies *in vitro*, 57
response to vitamin C, 92

Vagina, bismuth melanosis, 293
Vitamin—endocrine relationship
B and adrenals, 1
androgens, 97
hyperthyroidism 327, 433
C and adrenal cortex, 103
gonadotropin effect on testis, 140
progesterone, 92